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(54) Title: REGULATION OF CELL PROLIFERATION AND DIFFERENTIATION USING TOPICALLY APPLIED NUCLEIC ACID MOLECULES

(57) Abstract: Methods are disclosed for the regulation of cell differentiation and proliferation, e.g., for treating hyperproliferative skin disorder, such as psoriasis, and skin cancer for enhancing wound healing, for stimulating hair growth and inhibiting hair growth, by administration of nucleic acid molecules encoding parathyroid hormone (PTH), parathyroid related peptide (PTHrP), or fragment, analog or derivative thereof, and salts thereof, encapsulated by particular liposomes or incorporated into a porous boicompatable matrix.

Regulation Of Cell Proliferation And Differentiation Using Topically Applied Nucleic Acid Molecules

Background of the Invention

Field of the Invention

This invention relates to the regulation of cell differentiation and proliferation, e.g., for treating hyperproliferative skin disorder, such as psoriasis, for enhancing wound healing, for stimulating hair growth, and inhibiting hair growth by topical administration of nucleic acid molecules encoding parathyroid hormone (PTH), parathyroid related peptide (PTHrP), or a fragment or analog thereof.

Related Art

U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618 describe methods of inhibiting proliferation and enhancing differentiation of mammalian cells, inducing proliferation of mammalian cells, enhancing wound healing, and stimulating hair growth using a peptide which has a 10% or greater homology to a region of human PTH or human PTHrP. Certain fragments and analogs (e.g. PTH (1-34), PTH (3-34) and PTHrP (1-34)) were found to act as agonists of PTH and PTHrP and inhibit proliferation and enhance differentiation of mammalian cells. Other fragments and analogs (e.g. PTH (7-34) and PTHrP (7-34) are antagonists of PTH and PTHrP were also found to enhance the proliferation of mammalian cells. The agonists are useful for treatment of hyperproliferative skin diseases such a psoriasis, actinic keratoses, and skin cancer and the antagonists are useful for wound healing, particularly wounds of the skin, enhancing or maintaining hair growth, particularly following chemotherapeutic treatment of a mammal, and stimulating epidermal regrowth. Methods of administration include oral, nasal, intravenous, topical, subcutaneous, parenteral and intraperitoneal administration. The peptides may

be administered by subcutaneous pumps, patches, tapes, or by liposomal carriers.

A variety of PTH and PTHrP analogs and derivatives thereof have been made. See U.S. Pat. Nos. 4,086,196, 4,423,037, 4,771,124, 4,833,125, 4,968,669, 5,001,223, 5,087,562, 5,093,233, 5,116,952, 5,149,779, 5,171,670, 5,229,489, 5,317,010, 5,382,658, 5,393,869, 5,434,246, 5,527,772, 5,589,452, 5,807,823, 5,821,255, 5,840,690, 5,977,070, 6,025,467, 6,051,868, and 6,066,618; WO94/02510, WO00/23594, and WO00/31137; and EP 477,885. Methods for determining whether a particular analog is an agonist or antagonist of PTH and PTHrP are described in U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618.

Active vitamin D compounds are useful for treating hyperproliferative skin diseases and other conditions. A large number of such active vitamin D compounds are known. See U.S. Patent Nos. 5,457,217, 5,414,098, 5,384,313, 5,373,004, 5,371,249, 5,430,196, 5,260,290, 5,393,749, 5,395,830, 5,250,523, 5,247,104, 5,397,775, 5,194,431, 5,281,731, 5,254,538, 5,232,836, 5,185,150, 5,321,018, 5,086,191, 5,036,061, 5,030,772, 5,246,925, 4,973,584, 5,354,744, 4,927,815, 4,857,518, 4,851,401, 4,851,400, 4,847,012, 4,755,329, 4,940,700, 4,619,920, 4,594,192, 4,588,716, 4,564,474, 4,552,698, 4,588,528, 4,719,204, 4,719,205, 4,689,180, 4,505,906, 4,769,181, 4,502,991, 4,481,198, 4,448,726, 4,448,721, 4,428,946, 4,411,833, 4,367,177, 4,336,193, 4,360,472, 4,360,471, 4,307,231, 4,307,025, 4,358,406, 4,305,880, 4,279,826, and 4,248,791.

Summary of the Invention

The invention provides two important therapeutic methods one involving inhibition of cell proliferation and enhancement of skin cell differentiation (the agonist activity), which is useful in the treatment of psoriasis, ichthyosis, actinic keratoses, skin cancer, inhibiting hair growth or preventing hair regrowth. A second method involves enhancement of cell proliferation (the antagonist activity), which is useful in wound healing,

stimulating epidermal regrowth and hair growth. In addition, the invention provides methods for enhancing wound healing and hair growth based on in vivo wound healing activity or in vitro or in vivo hair growth activity rather than strict agonist or antagonist activity in vitro.

The first method of the invention generally involves inhibiting proliferation and enhancing differentiation of mammalian skin cells by contacting the cell with a nucleic acid molecule encoding a peptide which is preferably at least 3, and more preferably at least 8, amino acids long and has 10% or greater (more preferably, 50% or greater, and most preferably 75% or greater) sequence identity with a region (preferably within the amino-terminal 34 amino acid region) of human PTH or human PTHrP and, when expressed, is capable of inhibiting proliferation or enhancing the differentiation in vitro of cultured human keratinocytes; or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair growth. In preferred embodiments of this method, the peptide encoded by the nucleic acid molecule is hPTH (1-84), hPTH (1-34), hPTHrP (1-31), hPTHrP (1-40), hPTH (1-44), hPTH (1-36), hPTH (1-38), hPTH (1-31), hPTH (3-34), hPTHrP (1-34), hPTHrP (1-141), hPTHrP (1-139) or hPTHrP (1-173). This method has particular application in the treatment of hyperproliferative skin disorders such as psoriasis. The method may also be useful in the treatment of certain preskin cancers and skin cancers, by the inhibition of cancer cell proliferation and by the induction of differentiation and inhibition of hair growth or preventing hair growth and acne.

The second method of the invention generally involves enhancing proliferation of mammalian skin cells by contacting the skin cells with a nucleic acid molecule encoding a peptide which is preferably at least 3, and more preferably at least 8, amino acids long and has 10% or greater (more preferably, 50% or greater, and most preferably 75% or greater) sequence identity with a region (preferably within the amino-terminal 34 amino acid region) of hPTH or hPTHrP and, when expressed, is capable of blocking the differentiation or the inhibition of proliferation in vitro of cultured human

keratinocytes by PTH (1-34) or $1,25(OH)_2D_3$ or PTHrP (1-34); or *in vivo* in mouse skin by stimulating skin cell proliferation or accelerating hair cycle progression or stimulating hair growth. In a preferred embodiment of this method, the peptide encoded by the nucleic acid molecule is PTH (7-34), PTH (7-84), hPTH (5-34), hPTHrP (7-34), hPTHrP (5-34), hPTHrP (7-141), hPTHrP (7-134), or hPTHrP (7-173). In a related method of the invention, proliferation of mammalian skin cells, e.g., during wound healing, is enhanced by contacting the cell or wound with nucleic acid molecule encoding a peptide which is preferably at least 3, and more preferably at least 8, amino acids long and has 10% or greater (more preferably, 50% or greater, and most preferably, 75% or greater) sequence identity with a region (preferably, within the amino-terminal 34 amino acid region) of hPTH or hPTHrP, and, when expressed, is capable of enhancing wound healing in an in vivo skin punch assay. In preferred embodiments of this method, the peptide encoded by the nucleic acid molecule is hPTH (1-84), hPTH (1-34), hPTH (7-34), hPTH (5-34), hPTH (5-36), hPTH (1-31), hPTHrP (1-34), hPTHrP (1-135), hPTHrP (1-141), hPTHrP (1-173) or hPTHrP (7-34). These related methods have particular application in the enhancement of wound healing and also have applications in the promotion of skin growth in patients with burns or skin ulcerations as well as in the stimulation of epidermal regrowth in people who have decreased epidermal cell proliferation due to aging.

Hair growth is stimulated by administering to a mammal a nucleic acid molecule encoding a peptide which is preferably at least 3, and more preferably at least 8, amino acids long and has 10% or greater (more preferably, 50% or greater, and most preferably, 75% or greater) sequence identity with a region (preferably, within the amino-terminal 34 amino acid region) of hPTH or hPTHrP, and, when expressed, is capable of stimulating hair growth *in vitro* or *in vivo*. In preferred embodiments of this method, the peptide encoded by the nucleic acid molecule is hPTH (7-34), hPTH (7-84), hPTHrP (7-134), hPTHrP (7-141), hPTHrP (7-173), hPTH (5-34), hPTHrP (7-34) or hPTH (5-36).

The nucleic acid molecules are administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier. In a preferred embodiment, the carrier is a liposome or gel. In another preferred embodiment the nucleic acid molecules are contained within a porous biocompatable matrix.

The invention also relates to a method of inhibiting proliferation or enhancing differentiation of a skin or hair cell of a mammal, comprising administering to the mammal in need thereof a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule of the invention and an active vitamin D compound, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth. The invention also relates to a composition comprising a nucleic acid molecule of the invention encapsulated within a liposome.

The invention also relates to a composition comprising a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule of the invention and an active vitamin D compound, optionally encapsulated within a liposome.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Figures

FIG. 1 depicts a bar graph showing the effect of transfecting PTHrP (1-141) and PTHrP (1-173) genes into cultured keratinocytes on ^{3}H-thymidine incorporation. Bar 1 represents the empty vector, Bar 2 represents the PTHrP

gene (1-141) transfected into cultured human keratinocytes, Bar 3 represents the PTHrP gene (1-173) transfected into cultured human keratinocytes.

FIGs. 2A-2C depict schematic representations of the cDNA structure of the PTHrP (1-139), PTHrP (1-141) and PTHrP (1-173) genes.

FIG. 3 depicts a schematic representation of the pACCMV.pLpa adenoviral expression vector.

FIG. 4 depicts the sequence of SEQ ID NO: 1.

FIG. 5 depicts the sequence of SEQ ID NO: 2.

FIG. 6 depicts the sequence of SEQ ID NO: 3.

FIG. 7 depicts the sequence of SEQ ID NO: 4.

FIG. 8 depicts the sequence of SEQ ID NO: 5.

FIG. 9 depicts the sequence of SEQ ID NO: 6.

FIG. 10 depicts the sequence of SEQ ID NO: 7.

FIG. 11 depicts the sequence of SEQ ID NO: 8.

FIG. 12 depicts the sequence of SEQ ID NO: 9.

FIG. 13 depicts the sequence of SEQ ID NO: 10.

FIG. 14 depicts the sequence of SEQ ID NO: 11.

FIG. 15 depicts the sequence of SEQ ID NO: 12.

FIG. 16 depicts the sequence of SEQ ID NO: 13.

FIG. 17 depicts the sequence of SEQ ID NO: 14.

FIG. 18 depicts the sequence of SEQ ID NO: 15.

FIG. 19 depicts the sequence of SEQ ID NO: 16.

FIG. 20 depicts the sequence of SEQ ID NO: 17.

FIG. 21 depicts the sequence of SEQ ID NO: 18.

FIG. 22 depicts the sequence of SEQ ID NO: 19.

FIG. 23 depicts the sequence of SEQ ID NO: 20.

FIG. 24 depicts the sequence of SEQ ID NO: 21.

FIG. 25 depicts the sequence of SEQ ID NO: 22.

FIG. 26 depicts the sequence of SEQ ID NO: 23.

FIG. 27 depicts the sequence of SEQ ID NO: 24.

FIG. 28 depicts the sequence of SEQ ID NO: 25.

FIG. 29 depicts the sequence of SEQ ID NO: 26.

FIG. 30 depicts the sequence of SEQ ID NO: 27.

FIG. 31 depicts the sequence of SEQ ID NO: 28.

FIG. 32 depicts the sequence of SEQ ID NO: 29.

FIG. 33 depicts the sequence of SEQ ID NO: 30.

FIG. 34 depicts the sequence of SEQ ID NO: 31.

FIG. 35 depicts the sequence of SEQ ID NO: 32.

FIG. 36 depicts the sequence of SEQ ID NO: 33.

FIG. 37 depicts the sequence of SEQ ID NO: 34.

FIG. 38 depicts the sequence of SEQ ID NO: 35.

FIG. 39 depicts the sequence of SEQ ID NO: 36.

FIG. 40 depicts the sequence of SEQ ID NO: 37.

FIG. 41 depicts the sequence of SEQ ID NO: 38.

FIG. 42 depicts the sequence of SEQ ID NO: 39.

FIG. 43 depicts the sequence of SEQ ID NO: 40.

FIG. 44 depicts the sequence of SEQ ID NO: 41.

FIG. 45 depicts the sequence of SEQ ID NO: 42.

FIG. 46 depicts the sequence of SEQ ID NO: 43.

FIG. 47 depicts the sequence of SEQ ID NO: 44.

Description of the Preferred Embodiments

Nucleic Acid Molecules of the Invention

The invention relates to the regulation of cell differentiation and proliferation by administration of nucleic acid molecules encoding parathyroid hormone (PTH), parathyroid hormone related protein (PTHrP), or a fragment or analog thereof. Particular nucleic acid molecules which can be used include those which encode the following peptides:

hPTH (1-84), encoded by nucleotides 1-252 of the nucleic acid molecule of SEQ ID NO: 1 (Kimura, T. *et al.*, *BBRC* 11:493 (1983); Fairwell, T. *et al.*, *Biochemistry* 22:691 (1983)).

hPTH (1-31), encoded by nucleotides 1-93 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (1-34), encoded by nucleotides 1-102 of the nucleic acid molecule of SEQ ID NO: 1.

HPTH (1-36), encoded by nucleotides 1-108 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (1-38), encoded by nucleotides 1-114 of the nucleic acid molecule of SEQ ID NO:1 (Heech, R. D. *et al.*, *Horm. Metab. Res.* 16:556 (1984)).

hPTH (1-44), encoded by nucleotides 1-132 of the nucleic acid molecule of SEQ ID NO:1 (Kimura T. *et al.*, *Biopolymers* 20:1823 (1981)).

hPTH (5-36), encoded by nucleotides 13-108 of the nucleic acid molecule of SEQ ID NO:1.

HPTH (7-34), encoded by nucleotides 19-102 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (13-34), encoded by nucleotides 40-102 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (28-48), encoded by nucleotides 82-144 of the nucleic acid molecule of SEQ ID NO: 1 (Rosenblatt, M. *et al.*, *Biochemistry* 16:2811 (1977)).

HPTH (7-84), encoded by nucleotides 19-252 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (53-84), encoded by nucleotides 157-252 of the nucleic acid molecule of SEQ ID NO:1 (Rosenblatt, M. *et al.*, *Endocrinology* 103:976 (1978)).

hPTH (64-84), encoded by nucleotides 190-252 of the nucleic acid molecule of SEQ ID NO:1.

hPTH (70-84), encoded by nucleotides 208-252 of the nucleic acid molecule of SEQ ID NO:1.

[Tyr^{1}]-hPTH (1-34), encoded by nucleotides 1-102 of the nucleic acid molecule of SEQ ID NO: 1, wherein the adenosine at position 2 is mutated to a cytosine.

[Tyr^{27}]-hPTH (27-48), encoded by nucleotides 79-144 of the nucleic acid molecule of SEQ ID NO: 1, wherein the adenosine at position 79 and the guanosine at position 81 are both mutated to a thymidine.

[Tyr^{63}]-hPTH (63-84), encoded by nucleotides 187-252 of the nucleic acid molecule of SEQ ID NO: 1, wherein the cytosine at position 187 is mutated to a thymidine.

[Tyr^{69}]-hPTH (69-84), encoded by nucleotides 205-255 of the nucleic acid molecule of SEQ ID NO: 1, wherein the guanosine at position 205 is mutated to a thymidine, and the guanosine at position 207 is mutated to either a thymidine or a cytosine.

PTH, Bovine (bPTH) (1-84), encoded by nucleotides 1-252 of the nucleic acid molecule of SEQ ID NO: 2.

bPTH (1-34), encoded by nucleotides 1-102 of the nucleic acid molecule of SEQ ID NO:2 (Tregear, G. W. *et al., Biochemistry* 16:2817 (1977)).

bPTH (3-34), encoded by nucleotides 7-102 of the nucleic acid molecule of SEQ ID NO: 2 (Lowrik, C. *et al., Cell Calcium* 6:311 (1985)).

PTHrP (1-31), encoded by nucleotides 1-93 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (1-40), encoded by nucleotides 1-120 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (5-36), encoded by nucleotides 13-108 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (7-34), encoded by nucleotides 19-102 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (7-139), encoded by nucleotides 19-417 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (7-141), encoded by nucleotides 19-423 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (7-173), encoded by nucleotides 19-519 of the nucleic acid molecule of SEQ ID NO: 3.

Rat PTH (rPTH) (1-84), encoded by nucleotides 1-252 of the nucleic acid molecule of SEQ ID NO: 4 (Heinrich, G. *et al., J. Biol. Chem.* 25:3320 (1984)).

In addition, nucleic acid molecules which encode the peptides and peptide derivatives disclosed in the following documents can also be used: U.S. Pat. Nos. 4,086,196, 4,423,037, 4,771,124, 4,833,125, 4,968,669, 5,001,223, 5,087,562, 5,093,233, 5,116,952, 5,149,779, 5,171,670, 5,229,489, 5,317,010, 5,382,658, 5,393,869, 5,434,246, 5,527,772, 5,589,452, 5,807,823, 5,821,255, 5,840,690, 5,977,070, 6,025,467, 6,051,868, and 6,066,618; WO94/02510, WO00/23594, and WO00/31137; and EP 477,885.

A typical design for constructing the PTH (7-34), (7-84), (7-141), and PTHrP (7-34), (7-139), and (7-173) fragment cDNAs is to place a ATG start codon upstream of the initial peptide codon of the individual fragments and to introduce a stop codon downstream of the final peptide codon of the individual fragments. Also, an endogenous peptide cleavage site will be introduces between the ATG start codon and the initial peptide codon of the individual fragments to avoid unwanted amino acids being introduced into the constructs.

When selecting a candidate nucleic acid molecule for a method of this invention, a preferred first step is to choose a nucleic acid molecule encoding a peptide which includes a fragment which has at least 10%, and more preferably 50% or greater, sequence identity with an 8 or greater amino acid long fragment within the amino terminal 34 amino acid region of hPTH or hPTHrP. The term "sequence identity" refers to a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. (See, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer*

Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H. & Lipton, D., *SIAM J Applied Math 48*:1073 (1988)). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H. & Lipton, D., *SIAM J Applied Math 48*:1073 (1988). Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., *et al., Nucleic Acids Research 12(i)*:387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al., J Molec Biol 215*:403 (1990)).

Therefore, as used herein, the term "identity" represents a comparison between a test and reference sequence. More specifically, reference test sequence is defined as any test sequence that is 10% or more identical to a reference sequence. As used herein, the term at least 10% identical to refers to percent identities from 10 to 99.99 relative to the reference sequence. Identity at a level of 10% or more is indicative of the fact that, assuming for exemplification purposes a test and reference sequence length of 100 amino acids, that no more than 90% (i.e., 90 out of 100) of the amino acids in the test sequence differ from that of the reference sequence. Such differences may be represented as point mutations randomly distributed over the entire length of the nucleotide or amino acid sequence of the invention or they may be clustered in one or more locations of varying length up to the maximum allowable amino acid difference. Differences are defined as nucleotide or amino acid substitutions, or deletions.

Because of the high degree of homology among human PTH and PTH of other species, PTH peptides encoded by nucleic acids from non-human as well as human sources can be used. Similarly, human PTHrP (1-139), (1-141) and (1-173) have a high degree of homology with PTHrP of other species; therefore, nucleic acids from non-human as well as human sources can be used in the methods of the invention involving PTHrP.

Candidate nucleic acid molecules may be tested for suitability as inhibitors of cell proliferation and enhancers of differentiation using cultured human keratinocytes, similar to the method for testing peptides described in U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618. Briefly, those nucleic acid molecules encoding peptides which inhibit proliferation and induce differentiation in cultured keratinocytes are those potentially useful as therapeutic agents in treating disorders, e.g., psoriasis and cancer, where suppression of cell proliferation is desired. Candidate nucleic acid molecules may be tested for suitability as enhancers of cell proliferation using cultured human keratinocytes or *in vivo* mouse model. Those peptides encoded by the nucleic acid molecules which block the effect of agonist peptides or $1,25(OH)_2D_3$ on cultured keratinocyte proliferation are those potentially useful as therapeutic agents in treating disorders, e.g., wounds, burns, or skin ulcerations, where maintenance or stimulating of cell proliferation is desired.

Candidate nucleic acid molecules may be tested for their ability to enhance wound healing by carrying out a skin punch biopsy test, as described in U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618.

Candidate peptides may be tested for suitability as stimulators of hair growth using an in vitro hair growth assay, as described in U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618. Those peptides encoded by the nucleic acid molecules which stimulate hair growth in vitro are those potentially useful for the stimulation of hair growth in vivo, e.g., for the stimulation or maintenance of hair growth during or following chemotherapy or to treat a form of alopecia, e.g., male and female pattern baldness.

Alternatively, *in vivo* assays may be carried out as described herein and similar to those described in Schilli, M.B. *et al., J. Invest. Dermatol. 108*:928-932 (1997); Holick, M.F., *et al., Proc. Natl. Acad. Sci. 91*:8014-8016 (1994); Paus, R. and Cotsarelis, G., *N. Engl. J. Med. 341*: 491-497 (1999); Paus, R., *et al. Laboratory Invest. 60*: 365-369 (1989) and U.S. Pat. App. No. 60/213,247.

Care should be taken when determining the correct nucleic acid molecule for use in the invention. Experiments have shown that when normal cultured human keratinocytes are transfected with plasmids containing PTHrP (1-141) or PTHrP (1-173) an unexpected enhancement of cell growth is seen, as measured by ^{3}H-thymidine incorporation into epidermal DNA (FIG. 1). These results are attributed to proteolysis of the full-length peptide. For this reason, all candidate nucleic acid molecules should be tested for the expected activity before use.

Gene Therapy

In this preferred embodiment of the invention, a nucleic acid molecule encoding a peptide with desired activity is incorporated into a polynucleotide construct suitable for introducing the nucleic acid molecule into cells of the animal to be treated, to form a transfection vector. The transfection vector is then introduced into selected target tissues of the cells of the animal *in vivo* using any of a variety of methods known to those skilled in the art. Alternatively, naked DNA may be transfected into the cells, with or without cationic lipids.

Techniques for the construction of transfection vectors containing inserts of desired nucleic acid sequences are well-known in the art, and are generally described in "Working Toward Human Gene Therapy," Chapter 28 in *Recombinant DNA, 2nd Ed.*, Watson, J.D. *et al.* (eds.), Scientific American Books: New York (1992), pp. 567-581, or Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York (1989).

Gene therapy approaches that may be used to deliver a nucleic acid molecule include injection of plasmid DNA (Horton, H.M., *et al.*, *Proc. Natl. Acad. Sci. USA 96(4)*:1553-1558 (1999)); transduction using adenoviral vectors (Waugh, J.M., *et al.*, *Proc. Natl. Acad. Sci. USA 96(3)*:1065-1070 (1999)); transduction using retrovial vectors (Axelrod, J.H., *et al.*, *Proc. Natl. Acad. Sci. USA 87*:5173-5177 (1990); Drumm, M.L., *et al.*, *Cell 62*:1227-1233 (1990); Krueger, G.G., *et al.*, *J. Invest. Dermatol. 112*:233-239 (1999); Palmer, T.D., *et al.*, *Blood 73*:438-445 (1989); and Rosenberg, S.A., *et al.*, *N. Eng. J. Med. 323*:570-578 (1990)); and gene transfer using liposomes (Mason, C.A.E., *et al.*, *Nature Medicine 5(2)*:176-182 (1999)). In addition, general methods for construction of gene therapy vectors and the introduction of such vectors into a mammal for therapeutic purposes may be obtained in the above-referenced publications, the disclosures of which are specifically incorporated herein by reference in their entirety. In one such general method, vectors containing nucleic acid sequences of the present invention are directly introduced into the cells or tissues of the mammal to be treated, preferably by topical application. Such an approach is generally referred to as "*in vivo*" gene therapy.

Alternatively, cells or tissues may be removed from the mammal to be treated and placed into culture according to methods that are well-known to one of ordinary skill in the art. Transfection vectors or naked DNA containing the genes for desired peptides may then be introduced into these cells or tissues by any of the methods described generally above for introducing isolated polynucleotides into a cell or tissue. After a sufficient amount of time to allow incorporation of the inserted DNA, the cells or tissues may then be re-inserted into the mammal to be treated. Since introduction of the nucleic acid molecule encoding the peptide is performed outside of the body of the mammal, this approach is generally referred to as "*ex vivo*" gene therapy. See U.S. Patent No. 5,399,346. Gene transfer through transfection of cells *ex vivo* can be performed by a variety of methods, including, for example, calcium phosphate precipitation,

diethylaminoethyl dextran, electroporation, lipofection, or viral infection. Such methods are well known in the art (see, for example, Sambrook *et al.*).

For both *in vivo* and *ex vivo* gene therapy, the nucleic acid molecule encoding the desired peptide of the invention may be operatively linked to a regulatory DNA sequence, or "promoter," to form a genetic construct as described above. This construct, containing both the promoter and the nucleic acid molecule encoding the peptide, may be subcloned into a suitable vector such as a plasmid, adenovirus vector, retrovirus vector, or the like, and introduced into the animal to be treated in an in vivo gene therapy approach, or into the cells or tissues of the mammal in an ex vivo approach.

Alternatively, the nucleic acid molecule of the invention may be operatively linked to a heterologous regulatory DNA sequence, or promoter, to form a genetic construct as described above. The heterologous regulatory sequence may be tissue specific. The vector containing the genetic construct is then directly introduced into the animal to be treated or into the cells or tissues of the animal, as described.

The term "operably linked", as used herein, denotes a relationship between a regulatory region (typically a promoter element, but may include an enhancer element) and the gene, whereby the transcription of the gene is under the control of the regulatory region.

The term "heterologous" means a DNA sequence not found in the native genome. That is, two nucleic acid elements are said to be "heterologous" if the elements are derived from two different genes, or alternatively, two different species. Thus, "heterologous DNA regulatory sequence" indicates that the regulatory sequence is not naturally ligated to the nucleic acid molecule selected for use in the invention.

The term "promoter" is used according to its art-recognized meaning. It is intended to mean the DNA region, usually upstream to the coding sequence of a gene, which binds RNA polymerase and directs the enzyme to the correct transcriptional start site.

In general, a promoter may be functional in a variety of tissue types and in several different species of organisms, or its function may be restricted to a particular species and/or a particular tissue. Further, a promoter may be constitutively active, or it may be selectively activated by certain substances (e.g., a tissue-specific factor), under certain conditions (e.g., in the presence of an enhancer element, if present, in the genetic construct containing the promoter), or during certain developmental stages of the organism (e.g., active in fetus, silent in adult).

Promoters useful in the practice of the present invention are preferably "tissue-specific"--that is, they are capable of driving transcription of a gene in one tissue while remaining largely "silent" in other tissue types. Examples of tissue-specific promoters in the skin are the Keratin promoter (Vassar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 86:8565 (1989)), the POMC promoter (Deen *et al. Mol. Biol. Evol.* 9:483 (1992)), the alpha-actin promoter (Shani, *Mol. Cell. Biol.*, 6:2624 (1986)), the elastase-q promoter (Swift *et al., Cell* 28:639 (1984)), the tyrosine hydroxylase promoter (Kim, L. S., *et al., J. Biol. Chem 268*:15689 (1993); Kaneda, N., *et al., Neuron 6*:583 (1991)), the dopamine beta-hydroxylase promoter (Mercer E. H., *et al., Neuron 7*:703 (1991); Hcyle, G. W., *et al., J. Neurosci. 14*:2455 (1994)), the tryptophan hydroxylase promoter (Boularand, S., *et al., J. Biol. Chem 270*:3757 (1995); Stoll, J. and Goldman, D., *J. Neurosci. Res. 28*:457 (1991)) and the parathyroid hormone-related peptide promoter (Campos, R. V., *et al., Mol. Rnfovtinol. 6*:1642). For additional examples of tissue-specific promoters, see U.S. Patent Nos. 5,834,306 and 5,416,027, and references cited therein.

In addition to a promoter, the genetic construct may also contain other genetic control elements, such as enhancers, repressible sequences, and silencers, which may be used to regulate replication of the vector in the target cell. The only requirement is that the genetic element be activated, derepressed, enhanced, or otherwise genetically regulated by factors in the host cell and, with respect to methods of treatment, not in the non-target cell.

An "element," when used in the context of nucleic acid constructs, refers to a region of the construct or a nucleic acid fragment having a defined function.

For example, an enhancer element, as used herein, is a region of DNA that, when associated with inserted nucleic acid molecule, operably linked to a promoter, enhances the transcription of that gene.

The term "enhancer" is used according to its art-recognized meaning. It is intended to mean a sequence found in eukaryotes which can increase transcription from a gene when located (in either orientation) up to several kilobases from the gene being studied. These sequences usually act as enhancers when on the 5′ side (upstream) of the gene in question. However, some enhancers are active when placed on the 3′ side (downstream) of the gene. In some cases, enhancer elements can activate transcription from a gene with no (known) promoter.

Preferred enhancers include the DF3 breast cancer-specific enhancer and enhancers from viruses and the steroid receptor family. Other preferred transcriptional regulatory sequences include NF1, SP1, AP1, and FOS/JUN.

Any of a variety of methods known to those skilled in the art may be used to introduce transfection vectors of the present invention into selected target tissue cells. Such methods include, for example, viral-mediated gene transfer using retroviruses, adeno-associated virus (AAV), herpes virus, vaccinia virus, or RNA viruses (*e.g.*, Grunhaus and Horowitz, *Semin. Virol.* *3*:237-252 (1992); Herz and Gerard, *Proc. Nat. Acad. Sci. USA 90*:2812-2816 (1993); and Rosenfeld *et al.*, *Cell 68*:143-155 (1992)); liposome-mediated gene transfer (Morishita *et al.*, *J. Clin. Invest. 91*:2580 (1993); Felgner *et al.*, U.S. Patent Nos. 5,703,055 (1997) and 5,858,784 (1999)); injection of naked DNA directly into a target tissue (*e.g.*, Felgner *et al.*, U.S. Patent No. 5,589,466 (1996); Wolff *et al.*, U.S. Patent No. 5,693,622 (1997)); and receptor-mediated gene transfer (Wu and Wu, *Biochemistry 27*:887-892 (1988); Wagner *et al.*, *PNAS USA 87*:3410-3414 (1990); Curiel *et al.*, U.S. Patent 5,547,932 (1996); and Beug *et al.*, U.S. Patent No. 5,354,844 (1994)).

In any of these methods, where a vector may be targeted to selectively transfect a specific population of cells, it will be understood that in addition to local administration (such as may be achieved by injection into the target tissue), the vector may be administered systemically (e.g., intravenously) in a biologically-compatible solution or pharmaceutically acceptable delivery vehicle. Vector constructs administered in this way may selectively infect the target tissue. According to the present invention, the presence of a target tissue-specific promoter on the construct provides an independent means of restricting expression of the therapeutic gene.

Nucleic acid molecules encoding peptides which block antiproliferative compounds can also be useful in conjunction with chemotherapeutic agents in the treatment of skin cancer; many chemotherapeutic agents are effective only against dividing cells, and the blocking peptides can have the effect of inducing division of otherwise dormant cells, rendering them vulnerable to the chemotherapy. Nucleic acids encoding blocking peptides can also be useful in promoting growth of new cells, e.g., skin cells, in topical skin creams. Differentiation-inducing peptides can be used as immunostimulants, by inducing maturation of monocytes and lymphocytes bearing PTH receptors, while blocking peptides can be used to inhibit lymphocyte maturation, and thus can be used to treat conditions, e.g., autoimmune diseases such as juvenile diabetes, rheumatoid arthritis, and allograft rejection, where mature lymphocytes are a causative agent.

The nucleic acid molecules of the invention can be admixed with a pharmacologically inert topical carrier such as one comprising a gel, an ointment or a cream, including such carriers as water, glycerol, alcohol, propylene glycol, fatty alcohol, triglycerides, fatty acid ester or mineral oils. Other possible carriers are liquid petrolatum, isopropylpalmitate, polyethylene glycol ethanol 95%, polyoxyethylene monolaurate 5% in water, sodium lauryl sulfate 5% in water, and the like. Materials such as antioxidants, humectants, viscosity stabilizers and the like may be added, if necessary. Nucleic acid molecules can be incorporated into liposomes using methods outlined in U.S. Pat. 5,260,065.

The nucleic acid molecules can be incorporated into a collagenous biocompatable matrix similar to the methods utilized in Fang *et al., Proc. Nat. Acad. Sci. U.S.A.* 93:5753 (1996) and U.S. Pat. 5,962,427. The types of matrices that may be used in the practice of the invention is virtually limitless and may include both biological and synthetic matrices. The matrices may be biodegradable or non-biodegradable. The matrices may take the form of sponges, implants, tubes, telfa pads, band-aids, bandages, pads, lyophylized components, gels, patches, powders or nanoparicles. Particular examples of such matrices include porous or collagenous materials (*e.g.* type II collagen), hydroxyapatite, bioglass, aluminates, bioceramic materials, purified proteins or extracellular matrix compositions as well as metals such as titanium.

The nucleic acid molecules can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those of therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as hydrohalic acids, e.g, hydrochloric acid, sulfuric acid, or phsophoric acid.

Dosage will be dependent upon the age, health, and weight of the recipient; kind of concurrent treatment, if any; frequency of treatment; and the nature of the effect desired. Generally, daily dosage may be 0.001 to 500 μg/kg. The topical dosage may be from 0.01 to 100 $\mu g/cm^2$. The liposomal gel, ointment or cream formulations may be applied by one or more applications per day.

The invention also relates to compositions comprising a nucleic acid molecule of the invention, an active vitamin D compound and a pharmaceutical carrier, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when

expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair growth. A large number of active vitamin D compounds are known which can be used in the practice of the present invention. See U.S. Patent Nos. 5,457,217, 5,414,098, 5,384,313, 5,373,004, 5,371,249, 5,430,196, 5,260,290, 5,393,749, 5,395,830, 5,250,523, 5,247,104, 5,397,775, 5,194,431, 5,281,731, 5,254,538, 5,232,836, 5,185,150, 5,321,018, 5,086,191, 5,036,061, 5,030,772, 5,246,925, 4,973,584, 5,354,744, 4,927,815, 4,857,518, 4,851,401, 4,851,400, 4,847,012, 4,755,329, 4,940,700, 4,619,920, 4,594,192, 4,588,716, 4,564,474, 4,552,698, 4,588,528, 4,719,204, 4,719,205, 4,689,180, 4,505,906, 4,769,181, 4,502,991, 4,481,198, 4,448,726, 4,448,721, 4,428,946, 4,411,833, 4,367,177, 4,336,193, 4,360,472, 4,360,471, 4,307,231, 4,307,025, 4,358,406, 4,305,880, 4,279,826, and 4,248,791. A preferred active vitamin D compound is calcipotriene. In this embodiment, any conventional liposome may be used including the liposomes described in U.S. Pat. Nos. 4,235,871, 4,241,046, 4,247,411, 4,356,167, 4,377,567, 4,544,545, 4,551,288, 4,610,868, 4,731,210, 4,744,989, 4,772,471, 4,897,308, 4,917,951, 5,021,200, 5,032,457, and 5,260,065.

The invention relates as well to a method of inhibiting proliferation or enhancing differentiation of a skin or hair cell of a mammal, comprising administering to the mammal in need thereof a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule of the invention and an active vitamin D compound, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth. In this embodiment, the nucleic acid molecule encoding the peptide

and the active vitamin D compound may be administered as part of single or separate pharmaceutical compositions. Either one or both of the nucleic acid molecules and active vitamin D compound may be administered topically or parenterally. In a preferred embodiment, the nucleic acid molecule is administered first followed by the active vitamin D compound.

The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in clinical therapy and which are obvious to those skilled in the art are within the spirit and scope of the invention.

Example 1

Mini-gene construction

PTHrP gene: PTHrP gene expresses three isoform peptides: PTHrP 1-139, PTHrP1-141 and PTHrP 1-173. The gene splicing happens between exon 4 to exon 6. The 5'-flanking regions share common nucleotide sequences, including precursor peptide. PTHrP mini-genes were made based on the nucleotide sequences of the human PTHrP/PLP gene, (Yasuda *et al. J. Biol. Chem.* 264:7720 (1989)) by using the PCR technique. The interested gene fragments were constructed into pCR3.1eukaryotic expression vector. The forward primer for PTHrP (1-139), PTHrP (1-141), PTHrP (1-173) and PTHrP (1-34) is 5'-AGCGGAGACGATGCAGCGGAGA-3' (SEQ ID NO: 26), reverse primer for PTHrP (1-139) is 5'-AAGGGAGGCAGCTGAGACG-3' (SEQ ID NO: 27), for PTHrP (1-141) is 5'-GTCCTTGGAAGGTCTCTGCTG-3' (SEQ ID NO: 28), for PTHrP (1-173) is 5'-TTCTAGTGCCACTGCCCATTG-3' (SEQ ID NO:29) and for PTHrP (1-34) is 5'-CTACTAAGCTGTGTGGATTTCTGCGAT-3' (SEQ ID NO:

30). PCR was performed at 94°C for 3 min initial denaturing, then followed by denaturing for 30 seconds at 94°C, annealing for 30 seconds at 60°C and extension for 1 min at 72°C, total 30 cycles, additional extension for 10 min at 72°C.

Adenovirus construction of PTHrP

The corresponding mature and fragment forms of PTH or PTHrP cDNAs (FIG. 2) can be subcloned into the adenovirus expression vector, pACCMV.pLpA (FIG. 3). Once the PTH and PTHrP inserts are subcloned and purified they are co-transfected with pJM17 in 293 cells, which contains essential elements of the adenovirus genome to replicate and produce recombinant virions. The virions isolated for the co-transfected 293 cells are infectious but don't have the capacity to replicated in other cell types except 293 cells with the pJM17 vector. The purified pACCMV.pLpA. PTHrP virion particles can then be used for gene transfer of the various PTHrPs cDNAs driven by the CMV promoter in culture and animals (Tomas C. Berker, *et al.* Methods of Cell Biology, Use of Recombinant Adenovirus for Metabolic Engineering of Mammalian Cells, Vol. 43, Chp 8; pg. 161-187, Academic Press Inc., San Diego, CA., USA. 1994).

Transfection

Keratinocytes were maintained in MCDB-153 medium. Cells in 24 well dishes at 50%-60% confluence were transfected with 1 μg/ml of PTHrP cDNA which was constructed into pCR3.1 vector (INVITROGEN, San Diego, CA., USA), empty vector as a control. For each transfection, 0.5 micrograms of DNA and 3 microliters of LIPOFECTAMINE were diluted in 50 microliters of serum free media, respectively, and then combined for a DNA/ Liposome

complexing incubation for 15 minute at room temperature. DNA/ Liposome complex was then incubated on the cells for 3 hours. After 3 hour of transfection, fresh media was added and cells were incubated for 21 hours.

^3H - Thymidine Incorporation

^{3}H-thymidine incorporation into DNA was used as an index of cell proliferation as described previously (Smith E.L. *et al. J. Invert. Dermatology* 86:709 (1986), Holick *et al. Proc. Nat. Acad. Sci. U.S.A.* 91:8014 (1994)). Twenty-four hours post transfection the medium was replaced with 0.5 ml of fresh basal medium containing [methyl-^{3}H]thymidine (New England Nuclear, Boston, MA) and incubated for 3 h at 37° C. ^{3}H-Thymidine incorporation into DNA was stopped by placing the 24-well plates on ice. Unincorporated ^{3}H-thymidine was then removed and the cells were washed three times with ice-cold phosphate-buffered saline. DNA labeled with ^{3}H-thymidine and other macromolecules were first precipitated with ice-cold 5% perchloric acid for 20 min and then extracted with 0.5 ml of 5% perchloric acid at 70 °C for 20 min. The radioactivity in the extracts was determined by a liquid scintillation counter. The results were expressed as percent of control.

Experiments have shown that when normal cultured human keratinocytes are transfected with plasmids containing PTHrP (1-141) or PTHrP (1-173) an unexpected enhancement of cell growth is seen, as measured by ^{3}H-thymidine incorporation into epidermal DNA (FIG. 1). These results may be due to proteolysis of the full length peptide.

Having now fully described this invention, it will be understood by those of ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any embodiment thereof. All patents, patent applications and publications cited herein are fully incorporated by

reference herein in their entirety.

What Is Claimed Is:

1. A method of inhibiting proliferation or enhancing differentiation of a mammalian skin or hair cell, said method comprising administering to the mammalian skin or hair cell in need of inhibited proliferation or enhanced differentiation with a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth.

2. The method of claim 1, wherein said nucleic acid molecule is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

3. The method of claim 2, wherein said carrier is a liposome.

4. The method of claim 1, wherein said nucleic acid molecule is contained within a porous biocompatable matrix.

5. The method of claim 1, wherein said peptide encoded by the nucleic acid molecule is PTH (1-34) (SEQ ID NO: 18), PTHrP (1-34) (SEQ ID NO: 31), PTH (1-84) (SEQ ID NO: 15), PTHrP (1-141) (SEQ ID NO: 32), PTHrP (1-139) (SEQ ID NO: 33) or PTHrP (1-173) (SEQ ID NO: 34).

6. The method of claim 1, wherein said nucleic acid molecule is one of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, or a fragment thereof.

7. The method of claim 1, wherein said nucleic acid molecule is administered topically to the mammalian skin or hair cells.

8. The method of claim 1, wherein said method is a method of inhibiting a hyperproliferative skin disorder.

9. The method of claim 8, wherein said hyperproliferative skin disorder is psoriasis, ichthyosis, eczema, acne, actinic keratosis, or skin cancer.

10. The method of claim 1, wherein said method is a method of inhibiting hair growth or preventing hair regrowth.

11. The method of claim 1, wherein said peptide encoded by the nucleic acid molecule has at least 75% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP.

12. The method of claim 1, further comprising administering to the mammalian hair or skin cell an effective amount of an active vitamin D compound.

13. The method of claim 12, wherein said active vitamin D compound is calcipotriene.

14. The method of claim 12, wherein said active vitamin D compound is 1,25-dihydroxyvitamin D_3.

15. The method of claim 12, wherein said active vitamin D compound is 19-nor-1,25-dihydroxyvitamin D_2.

16. The method of claim 12, wherein said active vitamin D compound is 19-nor-1,25-dihyroxyvitamin D_3.

17. The method of claim 12, wherein said nucleic acid molecule and active vitamin D compound are administered topically or parenterally.

18. The method of claim 1, wherein said nucleic acid molecule is operably linked to a promoter.

19. The method of claim 1, wherein said nucleic acid molecule is contained by a plasmid.

20. The method of claim 1, wherein said nucleic acid molecule is contained by a viral vector.

21. A method of inhibiting proliferation or enhancing differentiation of a skin or hair cell of a mammal, said method comprising administering to the mammal in need thereof a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule and an active vitamin D compound, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth.

22. The method of claim 21, wherein said nucleic acid molecule and said active vitamin D compound are administered as part of a single pharmaceutical composition.

23. The method of claim 21, wherein said nucleic acid molecule and said active vitamin D compound are administered as part of separate pharmaceutical compositions.

24. The method of claim 21, wherein said nucleic acid molecule is administered parentally.

25. The method of claim 21, wherein said active vitamin D compound is administered topically.

26. The method of claim 21, wherein said active vitamin D compound is administered orally.

27. The method of claim 21, wherein said nucleic acid molecule is encapsulated within a liposome.

28. The method of claim 21, wherein said nucleic acid molecule is contained within a porous biocompatable matrix.

29. A method of inducing proliferation of a mammalian skin or hair cell, said method comprising administering to the mammalian skin or hair cell in need of proliferation with a proliferation-inducing amount of a nucleic acid molecule, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of blocking the inhibition of proliferation or stimulation of differentiation in vitro

of cultured human keratinocytes by PTH (1-34), $1,25(OH)_2D_3$ or PTHrP (1-34), or *in vivo* in mouse skin by stimulating skin cell proliferation or accelerating hair cycle progression or stimulating hair cell growth.

30. The method of claim 29, wherein said nucleic acid molecule is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

31. The method of claim 30, wherein said carrier is a liposome.

32. The method of claim 29, wherein said nucleic acid molecule is contained within a porous biocompatable matrix.

33. The method of claim 29, which is a method of stimulating skin cell growth, rejuvenating aged skin, preventing skin wrinkles, treating skin wrinkles, enhancing wound healing, stimulating hair growth, maintaining hair growth, treating or preventing female or male pattern baldness, or treating chemotherapy induced alopecia.

34. The method of claim 29, which is a method of stimulating epidermal cell growth or hair follicle cell growth.

35. The method of claim 29, wherein said peptide encoded by the nucleic acid molecule is PTH (7-34) (SEQ ID NO: 35), PTHrP (7-34) (SEQ ID NO: 36), PTH (5-36) (SEQ ID NO: 37), PTHrP (5-36) (SEQ ID NO: 38), PTH (5-34) (SEQ ID NO: 39), PTHrP (5-34) (SEQ ID NO: 40), PTH (7-84) (SEQ ID NO: 12), PTHrP (7-139) (SEQ ID NO: 41), PTHrP (4-141) (SEQ ID NO: 42), or PTHrP (7-173) (SEQ ID NO: 43)

36. The method of claim 1, wherein said nucleic acid molecule is one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, or a fragment thereof.

37. A composition comprising a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule encapsulated within a liposome, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth.

38. The method of claim 37, wherein said nucleic acid molecule is contained within a porous biocompatable matrix.

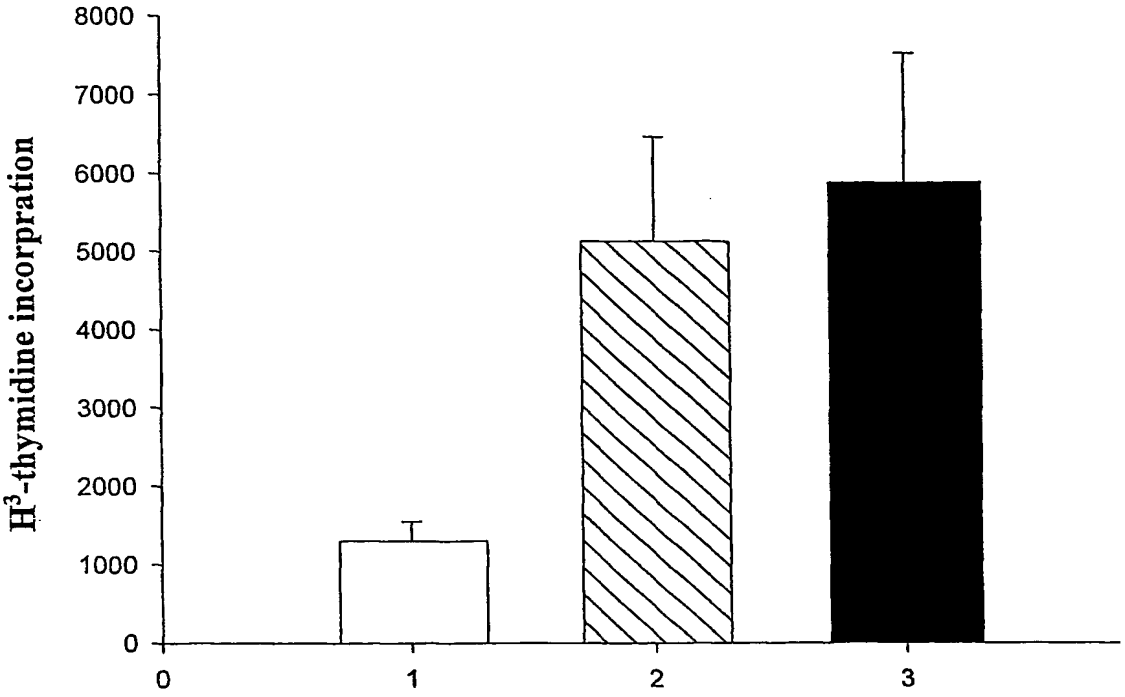
39. A composition comprising a proliferation-inducing amount of a nucleic acid molecule encapsulated within a liposome, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of blocking the inhibition of proliferation or stimulation of differentiation in vitro of cultured human keratinocytes by PTH (1-34), $1,25(OH)_2D_3$ or PTHrP (1-34), or *in vivo* in mouse skin by stimulating skin cell proliferation or accelerating hair cycle progression or stimulating hair cell growth.

40. A composition comprising a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule and an active vitamin D compound, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity

with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth.

41. The composition of claim 40, wherein at least one of said nucleic acid molecules or active vitamin D compound is encapsulated by liposomes.

FIG. 1


8000
7000
6000
5000
4000
3000
2000
1000
0
H^3-thymidine incorpration
0
1
2
3

PTHrP cDNA structure

FIG. 2A

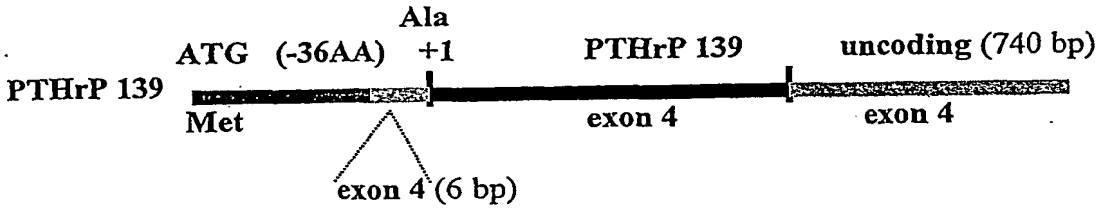


FIG. 2B

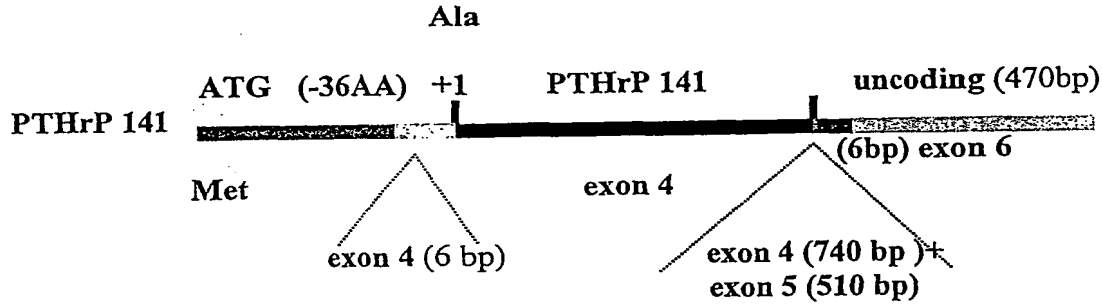
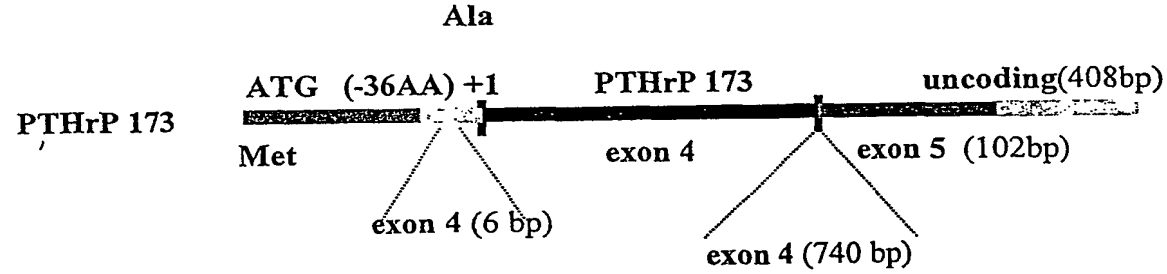


FIG 2C.



(Met – (-1AA) precursor peptide)

FIG. 3

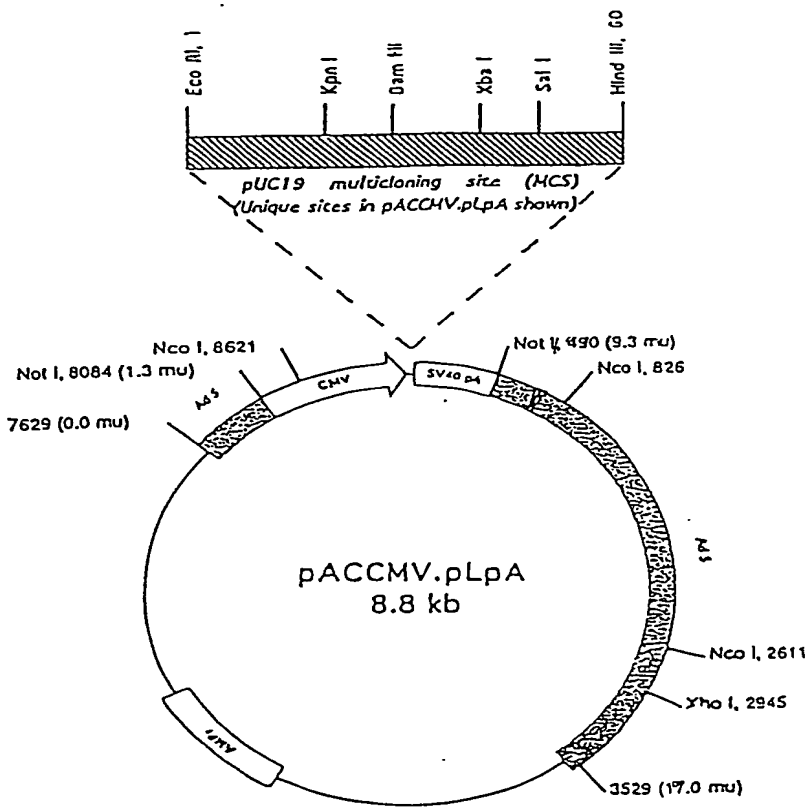

Eco RI, 1
Kpn I
Dam III
Xba I
Sal I
Hind III, 60
pUC19 multicloning site (MCS)
(Unique sites in pACCMV.pLpA shown)
Nco I, 8621
Not I, 8084 (1.3 mu)
7629 (0.0 mu)
CMV
SV40 pA
Not I, 490 (9.3 mu)
Nco I, 826
pACCMV.pLpA
8.8 kb
Nco I, 2611
Xho I, 2945
3529 (17.0 mu)

FIG. 4

SEQ ID NO: 1 - Human Parathyroid Hormone Coding Sequence (hPTH)

TCTGTGAGTGAAATACAGCTTATGCATAACCTGGGAAAACATCTGAACTC
GATGGAGAGAGTAGAATGGCTGCGTAAGAAGCTGCAGGATGTGCACAATT
TTGTTGCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTTCCCAGAGG
CCCCGAAAAAAGGAAGACAATGTCTTGGTTGAGAGCCATGAAAAAAGTCT
TGGAGAGGCAGACAAAGCTGATGTGAATGTATTAACTAAAGCTAAATCCC
AGTGA

FIG. 5

SEQ ID NO: 2 - Bovine Parathyroid Hormone Coding Sequence (bPTH)

GCTGTGAGTGAAATACAGTTTATGCATAACCTGGGCAAACATCTGAGCTC
CATGGAAAGAGTGGAATGGCTGCGGAAAAAGCTACAGGATGTGCACAACT
TTGTTGCCCTTGGAGCTTCTATAGCTTACAGAGATGGTAGTTCCCAGAGA
CCTCGAAAAAAGGAAGACAATGTCCTGGTTGAGAGCCATCAGAAAAGTCT
TGGAGAAGCAGACAAAGCTGATGTGGATGTATTAATTAAAGCTAAACCCC
AG

FIG. 6

SEQ ID NO: 3 - Human Parathyroid Hormone Related Protein Coding Sequence(PTHrP)

GCTGTGTCTGAACATCAGCTCCTCCATGACAAGGGGAAGTCCATCCAAGA
TTTACGGCGACGATTCTTCCTTCACCATCTGATCGCAGAAATCCACACAG
CTGAAATCAGAGCTACCTCGGAGGTGTCCCCTAACTCCAAGCCCTCTCCC
AACACAAAGAACCACCCCGTCCGATTTGGGTCTGATGATGAGGGCAGATA
CCTAACTCAGGAAACTAACAAGGTGGAGACGTACAAAGAGCAGCCGCTCA
AGACACCTGGGAAGAAAAAGAAAGGCAAGCCCGGGAAACGCAAGGAGCAG
GAAAAGAAAAAACGGCGAACTCGCTCTGCCTGGTTAGACTCTGGAGTGAC
TGGGAGTGGGCTAGAAGGGGACCACCTGTCTGACACCTCCACAACGTCGC
TGGAGCTCGATTCACGGAGGCATTGA

FIG. 7

SEQ ID NO: 4 - Rat Parathyroid Hormone Coding Sequence (rPTH)

GCTGTCAGTGAAATACAGCTTATGCACAACCTGGGCAAACACCTGGCCTC
TGTGGAGAGGATGCAATGGCTGAGAAAAAAGCTGCAAGATGTACACAATT
TTGTTAGTCTTGGAGTCCAAATGGCTGCCAGAGAAGGCAGTTACCAGAGG
CCCACCAAGAAGGAGGAAAATGTCCTTGTTGATGGCAATTCAAAAAGTCT
TGGCGAGGGGGACAAAGCTGATGTGGATGTATTAGTTAAGGCTAAATCTC
AGTAA

FIG. 8

SEQ ID NO:5 - hPTH (1-31) :

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDV

FIG. 9

SEQ ID NO:6 - PTHrP - (1-40)

H_2N-Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Phe-Phe-Leu-His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-Glu-Ile-Arg-Ala-Thr-Ser-OH

FIG. 10

SEQ ID NO:7 - PTH, Bovine (bPTH) (84 amino acids)

H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Ser-Ile-Ala-Tyr-Arg-Asp-Gly-Ser-Ser-Gln-Arg-Pro-Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Gln-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Ile-Lys-Ala-Lys-Pro-Gln-OH

FIG. 11

SEQ ID NO:8 - [Tyr^{63}]-hPTH (63-84)

H_2N-Tyr-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG. 12

SEQ ID NO:9 - hPTH (64-84)

H_2N-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG. 13

SEQ ID NO:10 - [Tyr^{69}]-hPTH (69-84)

H_2N-Tyr-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG. 14

SEQ ID NO:11 - hPTH (70-84)

H_2N -Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG. 15

SEQ ID NO:12 - hPTH (7-84)
H_2N-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-Ala-Gly-Ser-Gln-Arg-Pro-Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG. 16

SEQ ID NO:13 - hPTHrP (1-31)-

AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIH

FIG. 17

SEQ ID NO:14 - hPTH (1-34)

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF

FIG. 18

SEQ ID NO:15 - hPTH (84 amino acids)
H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-Ala-Gly-Ser-Gln-Arg-Pro-Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH (Kimura, T. et al, (1983) BBRC 114493; Fairwell, T. et al, (1983) Biochemistry 222691)

FIG. 19

SEQ ID NO:16 - Rat PTH (rPTH) (84 amino acids)
H_2N-Ala-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ala-Ser-Val-Glu-Arg-Met-Gln-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ser-Leu-Gly-Val-Gln-Met-Ala-Ala-Arg-Glu-Gly-Ser-Tyr-Gln-Arg-Pro-Thr-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Asp-Gly-Asn-Ser-Lys-Ser-Leu-Gly-Glu-Gly-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Val-Lys-Ala-Lys-Ser-Gln-OH (Heinrich, G. et al, (1984) J. Biol. Chem. 2593320)

FIG. 20

SEQ ID NO:17 - bPTH (1-34)
H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH (Tregear, G. W. et al, (1977) Biochemistry 162817)

FIG. 21

SEQ ID NO:18 - hPTH (1-34)
H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH (Takel, T. et al, (1979) Peptide Chemistry)

FIG. 22

SEQ ID NO:19 - [Tyr^1]-hPTH (1-34)
H_2N-Tyr-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH

FIG. 23

SEQ ID NO:20 - hPTH (1-38)
H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-OH (Heech, R. D. et al, (1984) Horm. Metab. Res. 16:556)

FIG. 24

SEQ ID NO:21 - bPTH (3-34)
H_2N-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH (Lowrik, C. et al, (1985) Cell Calcium 6:311)

FIG. 25

SEQ ID NO:22 - hPTH (13-34)
H_2N-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH

FIG. 26

SEQ ID NO:23 - [Tyr^{27}]-hPTH (27-28)
H_2N-Tyr-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-Ala-Gly-Ser-OH

FIG. 27

SEQ ID NO:24 - hPTH (28-48)
H_2N-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-Ala-Gly-Ser-OH Rosenblatt, M. et al, (1977) Biochemistry 16:2811)

FIG. 28

SEQ ID NO:25 - hPTH (53-84)
H_2N-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH
(Rosenblatt, M. et al, (1978) Endocrinology 103:976)

FIG. 29

SEQ ID NO: 26 - Oligo -
5'-AGCGGAGACGATGCAGCGGAGA-3'

FIG. 30

SEQ ID NO: 27 - Oligo -
5'-AAGGGAGGCAGCTGAGACG-3'

FIG. 31

SEQ ID NO: 28 - Oligo -
5'-GTCCTTGGAAGGTCTCTGCTG-3'

FIG. 32

SEQ ID NO: 29 - Oligo -
5'-TTCTAGTGCCACTGCCCATTG-3'

FIG. 33

SEQ ID NO: 30 - Oligo -
5'-CTACTAAGCTGTGTGGATTTCTGCGAT-3'

FIG. 34

SEQ ID NO: 31 - hPTHrP (1-34)-
AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTAE

FIG. 35

SEQ ID NO: 32 - hPTHrP (1-141) -
AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRFGSDDEGR
YLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDSGVTGSGLEGD
HLSDTSTTSLELDSRRH

FIG. 36

SEQ ID NO: 33 - hPTHrP (1-139) -
AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRFGSDDEGR
YLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDSGVTGSGLEGD
HLSDTSTTSLELDSR

FIG. 37

SEQ ID NO: 34 - hPTHrP (1-173) -
Met-Gln-Arg-Arg-Leu-Val-Gln-Gln-Trp-Ser-Val-Ala-Val-Phe-Leu

Leu-Ser-Tyr-Ala-Val-Pro-Ser-Cys-Gly-Arg-Ser-Val-Glu-Gly-Leu

Ser-Arg-Arg-Leu-Lys-Arg-Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His

Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Phe-Phe-Leu

His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-Glu-Ile-Arg-Ala-Thr

Ser-Glu-Val-Ser-Pro-Asn-Ser-Lys-Pro-Ser-Pro-Asn-Thr-Lys-Asn
His-Pro-Val-Arg-Phe-Gly-Ser-Asp-Asp-Glu-Gly-Arg-Tyr-Leu-Thr
Gln-Glu-Thr-Asn-Lys-Val-Glu-Thr-Tyr-Lys-Glu-Gln-Pro-Leu-Lys
Thr-Pro-Gly-Lys-Lys-Lys-Lys-Gly-Lys-Pro-Gly-Lys-Arg-Lys-Glu
Gln-Glu-Lys-Lys-Lys-Arg-Arg-Thr-Arg-Ser-Ala-Trp-Leu-Asp-Ser
Gly-Val-Thr-Gly-Ser-Gly-Leu-Glu-Gly-Asp-His-Leu-Ser-Asp-Thr
Ser-Thr-Thr-Ser-Leu-Glu-Leu-Asp-Ser-Arg-Thr-Ala-Leu-Leu-Trp
Gly-Leu-Lys-Lys-Lys-Lys-Glu-Asn-Asn-Arg-Arg-Thr-His-His-Met
Gln-Leu-Met-Ile-Ser-Leu-Phe-Lys-Ser-Pro-Leu-Leu-Leu-Leu-End

FIG. 38

SEQ ID NO: 35 - hPTH (7-34) -
LMHNLGKHLNSMERVEWLRKKLQDVHNF

Fig 39

SEQ ID NO: 36 - hPTHrP (7-34) -
LLHDKGKSIQDLRRRFFLHHLIAEIHTAE

Fig 40

SEQ ID NO: 37 - hPTH (5-36) -
IQLMHNLGKHLNSMERVEWLRKKLQDVHNFVA

FIG. 41

SEQ ID NO: 38 - hPTHrP (5-36)
H_2N-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-
Phe-Phe-Leu-His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-Glu-Ile -OH

FIG. 42

SEQ ID NO: 39 - hPTH (5-34)
H_2N-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-
Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH

FIG. 43

SEQ ID NO: 40 - hPTHrP (5-34)
H_2N-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-
Phe-Phe-Leu-His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-OH

FIG. 44

SEQ ID NO: 41 - hPTHrP (7-139) -
LLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRFGSDDEGR
YLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDSGVTGSGLEGD
HLSDTSTTSLELDSR

FIG. 45

SEQ ID NO: 42 - hPTHrP (7-141) -
LLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRFGSDDEGR
YLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDSGVTGSGLEGD
HLSDTSTTSLELDSRRH

FIG. 46

SEQ ID NO: 43 - hPTHrP (7-173) -
Gln-Gln-Trp-Ser-Val-Ala-Val-Phe-Leu

Leu-Ser-Tyr-Ala-Val-Pro-Ser-Cys-Gly-Arg-Ser-Val-Glu-Gly-Leu

Ser-Arg-Arg-Leu-Lys-Arg-Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His

Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Phe-Phe-Leu

His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-Glu-Ile-Arg-Ala-Thr

Ser-Glu-Val-Ser-Pro-Asn-Ser-Lys-Pro-Ser-Pro-Asn-Thr-Lys-Asn

His-Pro-Val-Arg-Phe-Gly-Ser-Asp-Asp-Glu-Gly-Arg-Tyr-Leu-Thr

Gln-Glu-Thr-Asn-Lys-Val-Glu-Thr-Tyr-Lys-Glu-Gln-Pro-Leu-Lys

Thr-Pro-Gly-Lys-Lys-Lys-Lys-Gly-Lys-Pro-Gly-Lys-Arg-Lys-Glu

Gln-Glu-Lys-Lys-Lys-Arg-Arg-Thr-Arg-Ser-Ala-Trp-Leu-Asp-Ser

Gly-Val-Thr-Gly-Ser-Gly-Leu-Glu-Gly-Asp-His-Leu-Ser-Asp-Thr

Ser-Thr-Thr-Ser-Leu-Glu-Leu-Asp-Ser-Arg-Thr-Ala-Leu-Leu-Trp

Gly-Leu-Lys-Lys-Lys-Lys-Glu-Asn-Asn-Arg-Arg-Thr-His-His-Met

Gln-Leu-Met-Ile-Ser-Leu-Phe-Lys-Ser-Pro-Leu-Leu-Leu-Leu-End

FIG. 47

SEQ ID NO: 44 - hPTH (1-44)

H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-OH (Kimura T. et al, (1981) Biopolymers 20:1823)

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<130> 1539.032PC00

<140>
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<150> US 60/238,134
<151> 2000-10-06

<160> 44

<170> PatentIn version 3.0

<210> 1
<211> 255
<212> DNA
<213> Human Parathyroid Hormone Coding Sequence (hPTH)

<400> 1
tctgtgagtg aaatacagct tatgcataac ctgggaaaac atctgaactc gatggagaga 60

gtagaatggc tgcgtaagaa gctgcaggat gtgcacaatt ttgttgccct tggagctcct 120

ctagctccca gagatgctgg ttcccagagg ccccgaaaaa aggaagacaa tgtcttggtt 180

gagagccatg aaaaaagtct tggagaggca gacaaagctg atgtgaatgt attaactaaa 240

gctaaatccc agtga 255

<210> 2
<211> 252
<212> DNA
<213> Bovine Parathyroid Hormone Coding Sequence (bPTH)

<400> 2
gctgtgagtg aaatacagtt tatgcataac ctgggcaaac atctgagctc catggaaaga 60

gtggaatggc tgcggaaaaa gctacaggat gtgcacaact ttgttgccct tggagcttct 120

atagcttaca gagatggtag ttcccagaga cctcgaaaaa aggaagacaa tgtcctggtt 180

gagagccatc agaaaagtct tggagaagca gacaaagctg atgtggatgt attaattaaa 240

gctaaacccc ag 252

<210> 3
<211> 426
<212> DNA
<213> Human Parathyroid Hormone Related Protein Coding Sequence (PTHrP)

<400> 3
gctgtgtctg aacatcagct cctccatgac aaggggaagt ccatccaaga tttacggcga 60

cgattcttcc ttcaccatct gatcgcagaa atccacacag ctgaaatcag agctacctcg 120

gaggtgtccc ctaactccaa gccctctccc aacacaaaga accaccccgt ccgatttggg 180

tctgatgatg agggcagata cctaactcag gaaactaaca aggtggagac gtacaaagag 240

cagccgctca agacacctgg gaagaaaaag aaaggcaagc ccgggaaacg caaggagcag 300

gaaaagaaaa aacggcgaac tcgctctgcc tggttagact ctggagtgac tgggagtggg 360

ctagaagggg accacctgtc tgacacctcc acaacgtcgc tggagctcga ttcacggagg 420

cattga 426

<210> 4
<211> 255
<212> DNA
<213> Rat Parathyroid Hormone Coding Sequence (rPTH)

<400> 4
gctgtcagtg aaatacagct tatgcacaac ctgggcaaac acctggcctc tgtggagagg 60

atgcaatggc tgagaaaaaa gctgcaagat gtacacaatt ttgttagtct tggagtccaa 120

atggctgcca gagaaggcag ttaccagagg cccaccaaga aggaggaaaa tgtccttgtt 180

gatggcaatt caaaaagtct tggcgagggg gacaaagctg atgtggatgt attagttaag 240

gctaaatctc agtaa 255

<210> 5
<211> 30
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<400> 5
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Leu Gln Asp Val
20 25 30

<210> 6
<211> 40
<212> PRT
<213> PTHrP - (1-40)

<400> 6
Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln
1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala Glu Ile Arg Ala Thr Ser
35 40

<210> 7
<211> 84

<212> PRT
<213> bPTH

<400> 7
Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe Val Ala Leu Gly Ala Ser Ile Ala Tyr Arg Asp Gly Ser Ser
35 40 45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Gln
50 55 60

Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asp Val Leu Ile Lys
65 70 75 80

Ala Lys Pro Gln

<210> 8
<211> 22
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<213> [Tyr^{63}] hPTH (63-84)

<400> 8
Tyr Glu Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asn Val Leu
1 5 10 15

Thr Lys Ala Lys Ser Gln
20

<210> 9
<211> 21
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<213> hPTH (64-84)

<400> 9
Glu Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr
1 5 10 15

Lys Ala Lys Ser Gln
20

<210> 10
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1 5 10 15

<210> 11
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<400> 11
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1 5 10 15

<210> 12
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<400> 12
Leu Met His Asn Leu Gly Lys His Leu Asn Ser Met Glu Arg Val Glu
1 5 10 15

Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu Gly
20 25 30

Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser Gln Arg Pro Arg Lys Lys
35 40 45

Glu Asp Asn Val Leu Val Glu Ser His Glu Lys Ser Leu Gly Glu Ala
50 55 60

Asp Lys Ala Asp Val Asn Val Leu Thr Lys Ala Lys Ser Gln
65 70 75

<210> 13
<211> 32
<212> PRT
<213> hPTHrP (1-31)

<400> 13
Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln
1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

<210> 14
<211> 34
<212> PRT
<213> hPTH (1-34)

<400> 14
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
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Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

<210> 15
<211> 84
<212> PRT
<213> hPTH

<400> 15
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn

1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser
35 40 45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu
50 55 60

Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr Lys
65 70 75 80

Ala Lys Ser Gln

<210> 16
<211> 84
<212> PRT
<213> rPTH

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Ala Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Ala
1 5 10 15

Ser Val Glu Arg Met Gln Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe Val Ser Leu Gly Val Gln Met Ala Ala Arg Glu Gly Ser Tyr
35 40 45

Gln Arg Pro Thr Lys Lys Glu Asp Asn Val Leu Val Asp Gly Asn Ser
50 55 60

Lys Ser Leu Gly Glu Gly Asp Lys Ala Asp Val Asp Val Leu Val Lys
65 70 75 80

Ala Lys Ser Gln

<210> 17
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<212> PRT
<213> bPTH (1-34)

<400> 17
Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

<210> 18
<211> 34
<212> PRT
<213> hPTH (1-34)

<400> 18

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

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<400> 19
Tyr Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe

<210> 20
<211> 38
<212> PRT
<213> hPTH (1-38)

<400> 20
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe Val Ala Leu Gly
35

<210> 21
<211> 32
<212> PRT
<213> bPTH (3-34)

<400> 21
Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser Ser Met
1 5 10 15

Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe
20 25 30

<210> 22
<211> 22
<212> PRT
<213> hPTH (13-34)

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Lys His Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu
1 5 10 15

Gln Asp Val His Asn Phe
20

<210> 23
<211> 22
<212> PRT
<213> [Tyr^{27}] hPTH (27-28)

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1 5 10 15

Pro Arg Asp Ala Gly Ser
20

<210> 24
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1 5 10 15

Arg Asp Ala Gly Ser
20

<210> 25
<211> 32
<212> PRT
<213> hPTH (53-84)

<400> 25
Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu Lys Ser Leu Gly
1 5 10 15

Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr Lys Ala Lys Ser Gln
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<212> DNA
<213> Oligonucleotide

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<212> DNA
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<400> 28
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<213> Oligonucleotide

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<212> DNA
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<400> 30
ctactaagct gtgtggattt ctgcgat 27

<210> 31
<211> 35
<212> PRT
<213> hPTHrP (1-34)

<400> 31
Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln
1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala Glu
35

<210> 32
<211> 141
<212> PRT
<213> hPTHrP (1-141)

<400> 32
Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln
1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala Glu Ile Arg Ala Thr Ser Glu Val Ser Pro Asn Ser Lys Pro
35 40 45

Ser Pro Asn Thr Lys Asn His Pro Val Arg Phe Gly Ser Asp Asp Glu
50 55 60

Gly Arg Tyr Leu Thr Gln Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu
65 70 75 80

Gln Pro Leu Lys Thr Pro Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys
85 90 95

Arg Lys Glu Gln Glu Lys Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu
100 105 110

Asp Ser Gly Val Thr Gly Ser Gly Leu Glu Gly Asp His Leu Ser Asp
115 120 125

Thr Ser Thr Thr Ser Leu Glu Leu Asp Ser Arg Arg His
130 135 140

<210> 33
<211> 139
<212> PRT
<213> hPTHrP (1-139)

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1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala Glu Ile Arg Ala Thr Ser Glu Val Ser Pro Asn Ser Lys Pro
35 40 45

Ser Pro Asn Thr Lys Asn His Pro Val Arg Phe Gly Ser Asp Asp Glu
50 55 60

Gly Arg Tyr Leu Thr Gln Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu
65 70 75 80

Gln Pro Leu Lys Thr Pro Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys
85 90 95

Arg Lys Glu Gln Glu Lys Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu
100 105 110

Asp Ser Gly Val Thr Gly Ser Gly Leu Glu Gly Asp His Leu Ser Asp
115 120 125

Thr Ser Thr Thr Ser Leu Glu Leu Asp Ser Arg
130 135

<210> 34
<211> 209
<212> PRT
<213> hPTHrP (1-173)

<400> 34
Met Gln Arg Arg Leu Val Gln Gln Trp Ser Val Ala Val Phe Leu Leu
1 5 10 15

Ser Tyr Ala Val Pro Ser Cys Gly Arg Ser Val Glu Gly Leu Ser Arg
20 25 30

Arg Leu Lys Arg Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly
35 40 45

Lys Ser Ile Gln Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile
50 55 60

Ala Glu Ile His Thr Ala Glu Ile Arg Ala Thr Ser Glu Val Ser Pro
65 70 75 80

Asn Ser Lys Pro Ser Pro Asn Thr Lys Asn His Pro Val Arg Phe Gly
85 90 95

Ser Asp Asp Glu Gly Arg Tyr Leu Thr Gln Glu Thr Asn Lys Val Glu
100 105 110

Thr Tyr Lys Glu Gln Pro Leu Lys Thr Pro Gly Lys Lys Lys Lys Gly
115 120 125

Lys Pro Gly Lys Arg Lys Glu Gln Glu Lys Lys Lys Arg Arg Thr Arg
130 135 140

Ser Ala Trp Leu Asp Ser Gly Val Thr Gly Ser Gly Leu Glu Gly Asp
145 150 155 160

His Leu Ser Asp Thr Ser Thr Thr Ser Leu Glu Leu Asp Ser Arg Thr
165 170 175

Ala Leu Leu Trp Gly Leu Lys Lys Lys Lys Glu Asn Asn Arg Arg Thr
180 185 190

His His Met Gln Leu Met Ile Ser Leu Phe Lys Ser Pro Leu Leu Leu
195 200 205

Leu

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<213> hPTH (7-34)

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1 5 10 15

Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe
20 25

<210> 36
<211> 29
<212> PRT
<213> hPTHrP (7-34)

<400> 36
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1 5 10 15

Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala Glu
20 25

<210> 37
<211> 32
<212> PRT
<213> hPTH (5-36)

<400> 37
Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn Ser Met Glu Arg
1 5 10 15

Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala
20 25 30

<210> 38
<211> 32
<212> PRT
<213> hPTHrP (5-36)

<400> 38
His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala Glu Ile
20 25 30

<210> 39
<211> 30
<212> PRT
<213> hPTH (5-34)

<400> 39
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1 5 10 15

Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe
20 25 30

<210> 40
<211> 30
<212> PRT
<213> hPTHrP (5-34)

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1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala
20 25 30

<210> 41
<211> 133
<212> PRT
<213> hPTHrP (7-139)

<400> 41
Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg Arg Phe
1 5 10 15

Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala Glu Ile Arg Ala
20 25 30

Thr Ser Glu Val Ser Pro Asn Ser Lys Pro Ser Pro Asn Thr Lys Asn
35 40 45

His Pro Val Arg Phe Gly Ser Asp Asp Glu Gly Arg Tyr Leu Thr Gln
50 55 60

Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu Gln Pro Leu Lys Thr Pro
65 70 75 80

Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys Arg Lys Glu Gln Glu Lys
85 90 95

Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu Asp Ser Gly Val Thr Gly
100 105 110

Ser Gly Leu Glu Gly Asp His Leu Ser Asp Thr Ser Thr Thr Ser Leu
115 120 125

Glu Leu Asp Ser Arg
130

<210> 42
<211> 135
<212> PRT
<213> hPTHrP (7-141)

<400> 42
Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg Arg Phe
1 5 10 15

Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala Glu Ile Arg Ala
20 25 30

Thr Ser Glu Val Ser Pro Asn Ser Lys Pro Ser Pro Asn Thr Lys Asn
35 40 45

His Pro Val Arg Phe Gly Ser Asp Asp Glu Gly Arg Tyr Leu Thr Gln
50 55 60

Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu Gln Pro Leu Lys Thr Pro
65 70 75 80

Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys Arg Lys Glu Gln Glu Lys
85 90 95

Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu Asp Ser Gly Val Thr Gly
100 105 110

Ser Gly Leu Glu Gly Asp His Leu Ser Asp Thr Ser Thr Thr Ser Leu
115 120 125

Glu Leu Asp Ser Arg Arg His
130 135

<210> 43
<211> 203
<212> PRT
<213> hPTHrP (7-173)

<400> 43
Gln Gln Trp Ser Val Ala Val Phe Leu Leu Ser Tyr Ala Val Pro Ser
1 5 10 15

Cys Gly Arg Ser Val Glu Gly Leu Ser Arg Arg Leu Lys Arg Ala Val
20 25 30

Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu

35 40 45

Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala
50 55 60

Glu Ile Arg Ala Thr Ser Glu Val Ser Pro Asn Ser Lys Pro Ser Pro
65 70 75 80

Asn Thr Lys Asn His Pro Val Arg Phe Gly Ser Asp Asp Glu Gly Arg
85 90 95

Tyr Leu Thr Gln Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu Gln Pro
100 105 110

Leu Lys Thr Pro Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys Arg Lys
115 120 125

Glu Gln Glu Lys Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu Asp Ser
130 135 140

Gly Val Thr Gly Ser Gly Leu Glu Gly Asp His Leu Ser Asp Thr Ser
145 150 155 160

Thr Thr Ser Leu Glu Leu Asp Ser Arg Thr Ala Leu Leu Trp Gly Leu
165 170 175

Lys Lys Lys Lys Glu Asn Asn Arg Arg Thr His His Met Gln Leu Met
180 185 190

Ile Ser Leu Phe Lys Ser Pro Leu Leu Leu Leu
195 200

<210> 44
<211> 44
<212> PRT
<213> hPTH (1-44)

<400> 44
Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn
1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg
35 40

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(54) Title: REGULATION OF CELL PROLIFERATION AND DIFFERENTIATION USING TOPICALLY APPLIED NUCLEIC ACID MOLECULES

(57) Abstract: Methods are disclosed for the regulation of cell differentiation and proliferation, e.g., for treating hyperproliferative skin disorder, such as psoriasis, and skin cancer for enhancing wound healing, for stimulating hair growth and inhibiting hair growth, by administration of nucleic acid molecules encoding parathyroid hormone (PTH), parathyroid related peptide (PTHrP), or fragment, analog or derivative thereof, and salts thereof, encapsulated by particular liposomes or incorporated into a porous boicompatable matrix.

BNSDOCID: <WO 02028420A2 IA>

Regulation Of Cell Proliferation And Differentiation Using Topically Applied Nucleic Acid Molecules

Background of the Invention

Field of the Invention

This invention relates to the regulation of cell differentiation and proliferation, e.g., for treating hyperproliferative skin disorder, such as psoriasis, for enhancing wound healing, for stimulating hair growth, and inhibiting hair growth by topical administration of nucleic acid molecules encoding parathyroid hormone (PTH), parathyroid related peptide (PTHrP), or a fragment or analog thereof.

Related Art

U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618 describe methods of inhibiting proliferation and enhancing differentiation of mammalian cells, inducing proliferation of mammalian cells, enhancing wound healing, and stimulating hair growth using a peptide which has a 10% or greater homology to a region of human PTH or human PTHrP. Certain fragments and analogs (e.g. PTH (1-34), PTH (3-34) and PTHrP (1-34)) were found to act as agonists of PTH and PTHrP and inhibit proliferation and enhance differentiation of mammalian cells. Other fragments and analogs (e.g. PTH (7-34) and PTHrP (7-34) are antagonists of PTH and PTHrP were also found to enhance the proliferation of mammalian cells. The agonists are useful for treatment of hyperproliferative skin diseases such a psoriasis, actinic keratoses, and skin cancer and the antagonists are useful for wound healing, particularly wounds of the skin, enhancing or maintaining hair growth, particularly following chemotherapeutic treatment of a mammal, and stimulating epidermal regrowth. Methods of administration include oral, nasal, intravenous, topical, subcutaneous, parenteral and intraperitoneal administration. The peptides may

be administered by subcutaneous pumps, patches, tapes, or by liposomal carriers.

A variety of PTH and PTHrP analogs and derivatives thereof have been made. See U.S. Pat. Nos. 4,086,196, 4,423,037, 4,771,124, 4,833,125, 4,968,669, 5,001,223, 5,087,562, 5,093,233, 5,116,952, 5,149,779, 5,171,670, 5,229,489, 5,317,010, 5,382,658, 5,393,869, 5,434,246, 5,527,772, 5,589,452, 5,807,823, 5,821,255, 5,840,690, 5,977,070, 6,025,467, 6,051,868, and 6,066,618; WO94/02510, WO00/23594, and WO00/31137; and EP 477,885. Methods for determining whether a particular analog is an agonist or antagonist of PTH and PTHrP are described in U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618.

Active vitamin D compounds are useful for treating hyperproliferative skin diseases and other conditions. A large number of such active vitamin D compounds are known. See U.S. Patent Nos. 5,457,217, 5,414,098, 5,384,313, 5,373,004, 5,371,249, 5,430,196, 5,260,290, 5,393,749, 5,395,830, 5,250,523, 5,247,104, 5,397,775, 5,194,431, 5,281,731, 5,254,538, 5,232,836, 5,185,150, 5,321,018, 5,086,191, 5,036,061, 5,030,772, 5,246,925, 4,973,584, 5,354,744, 4,927,815, 4,857,518, 4,851,401, 4,851,400, 4,847,012, 4,755,329, 4,940,700, 4,619,920, 4,594,192, 4,588,716, 4,564,474, 4,552,698, 4,588,528, 4,719,204, 4,719,205, 4,689,180, 4,505,906, 4,769,181, 4,502,991, 4,481,198, 4,448,726, 4,448,721, 4,428,946, 4,411,833, 4,367,177, 4,336,193, 4,360,472, 4,360,471, 4,307,231, 4,307,025, 4,358,406, 4,305,880, 4,279,826, and 4,248,791.

Summary of the Invention

The invention provides two important therapeutic methods one involving inhibition of cell proliferation and enhancement of skin cell differentiation (the agonist activity), which is useful in the treatment of psoriasis, ichthyosis, actinic keratoses, skin cancer, inhibiting hair growth or preventing hair regrowth. A second method involves enhancement of cell proliferation (the antagonist activity), which is useful in wound healing,

stimulating epidermal regrowth and hair growth. In addition, the invention provides methods for enhancing wound healing and hair growth based on in vivo wound healing activity or in vitro or in vivo hair growth activity rather than strict agonist or antagonist activity in vitro.

The first method of the invention generally involves inhibiting proliferation and enhancing differentiation of mammalian skin cells by contacting the cell with a nucleic acid molecule encoding a peptide which is preferably at least 3, and more preferably at least 8, amino acids long and has 10% or greater (more preferably, 50% or greater, and most preferably 75% or greater) sequence identity with a region (preferably within the amino-terminal 34 amino acid region) of human PTH or human PTHrP and, when expressed, is capable of inhibiting proliferation or enhancing the differentiation in vitro of cultured human keratinocytes; or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair growth. In preferred embodiments of this method, the peptide encoded by the nucleic acid molecule is hPTH (1-84), hPTH (1-34), hPTHrP (1-31), hPTHrP (1-40), hPTH (1-44), hPTH (1-36), hPTH (1-38), hPTH (1-31), hPTH (3-34), hPTHrP (1-34), hPTHrP (1-141), hPTHrP (1-139) or hPTHrP (1-173). This method has particular application in the treatment of hyperproliferative skin disorders such as psoriasis. The method may also be useful in the treatment of certain preskin cancers and skin cancers, by the inhibition of cancer cell proliferation and by the induction of differentiation and inhibition of hair growth or preventing hair growth and acne.

The second method of the invention generally involves enhancing proliferation of mammalian skin cells by contacting the skin cells with a nucleic acid molecule encoding a peptide which is preferably at least 3, and more preferably at least 8, amino acids long and has 10% or greater (more preferably, 50% or greater, and most preferably 75% or greater) sequence identity with a region (preferably within the amino-terminal 34 amino acid region) of hPTH or hPTHrP and, when expressed, is capable of blocking the differentiation or the inhibition of proliferation in vitro of cultured human

keratinocytes by PTH (1-34) or $1,25(OH)_2D_3$ or PTHrP (1-34); or *in vivo* in mouse skin by stimulating skin cell proliferation or accelerating hair cycle progression or stimulating hair growth. In a preferred embodiment of this method, the peptide encoded by the nucleic acid molecule is PTH (7-34), PTH (7-84), hPTH (5-34), hPTHrP (7-34), hPTHrP (5-34), hPTHrP (7-141), hPTHrP (7-134), or hPTHrP (7-173). In a related method of the invention, proliferation of mammalian skin cells, e.g., during wound healing, is enhanced by contacting the cell or wound with nucleic acid molecule encoding a peptide which is preferably at least 3, and more preferably at least 8, amino acids long and has 10% or greater (more preferably, 50% or greater, and most preferably, 75% or greater) sequence identity with a region (preferably, within the amino-terminal 34 amino acid region) of hPTH or hPTHrP, and, when expressed, is capable of enhancing wound healing in an in vivo skin punch assay. In preferred embodiments of this method, the peptide encoded by the nucleic acid molecule is hPTH (1-84), hPTH (1-34), hPTH (7-34), hPTH (5-34), hPTH (5-36), hPTH (1-31), hPTHrP (1-34), hPTHrP (1-135), hPTHrP (1-141), hPTHrP (1-173) or hPTHrP (7-34). These related methods have particular application in the enhancement of wound healing and also have applications in the promotion of skin growth in patients with burns or skin ulcerations as well as in the stimulation of epidermal regrowth in people who have decreased epidermal cell proliferation due to aging.

Hair growth is stimulated by administering to a mammal a nucleic acid molecule encoding a peptide which is preferably at least 3, and more preferably at least 8, amino acids long and has 10% or greater (more preferably, 50% or greater, and most preferably, 75% or greater) sequence identity with a region (preferably, within the amino-terminal 34 amino acid region) of hPTH or hPTHrP, and, when expressed, is capable of stimulating hair growth *in vitro* or *in vivo*. In preferred embodiments of this method, the peptide encoded by the nucleic acid molecule is hPTH (7-34), hPTH (7-84), hPTHrP (7-134), hPTHrP (7-141), hPTHrP (7-173), hPTH (5-34), hPTHrP (7-34) or hPTH (5-36).

The nucleic acid molecules are administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier. In a preferred embodiment, the carrier is a liposome or gel. In another preferred embodiment the nucleic acid molecules are contained within a porous biocompatable matrix.

The invention also relates to a method of inhibiting proliferation or enhancing differentiation of a skin or hair cell of a mammal, comprising administering to the mammal in need thereof a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule of the invention and an active vitamin D compound, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth. The invention also relates to a composition comprising a nucleic acid molecule of the invention encapsulated within a liposome.

The invention also relates to a composition comprising a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule of the invention and an active vitamin D compound, optionally encapsulated within a liposome.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Figures

FIG. 1 depicts a bar graph showing the effect of transfecting PTHrP (1-141) and PTHrP (1-173) genes into cultured keratinocytes on ^{3}H-thymidine incorporation. Bar 1 represents the empty vector, Bar 2 represents the PTHrP

gene (1-141) transfected into cultured human keratinocytes, Bar 3 represents the PTHrP gene (1-173) transfected into cultured human keratinocytes.

FIGs. 2A-2C depict schematic representations of the cDNA structure of the PTHrP (1-139), PTHrP (1-141) and PTHrP (1-173) genes.

FIG. 3 depicts a schematic representation of the pACCMV.pLpa adenoviral expression vector.

FIG. 4 depicts the sequence of SEQ ID NO: 1.

FIG. 5 depicts the sequence of SEQ ID NO: 2.

FIG. 6 depicts the sequence of SEQ ID NO: 3.

FIG. 7 depicts the sequence of SEQ ID NO: 4.

FIG. 8 depicts the sequence of SEQ ID NO: 5.

FIG. 9 depicts the sequence of SEQ ID NO: 6.

FIG. 10 depicts the sequence of SEQ ID NO: 7.

FIG. 11 depicts the sequence of SEQ ID NO: 8.

FIG. 12 depicts the sequence of SEQ ID NO: 9.

FIG. 13 depicts the sequence of SEQ ID NO: 10.

FIG. 14 depicts the sequence of SEQ ID NO: 11.

FIG. 15 depicts the sequence of SEQ ID NO: 12.

FIG. 16 depicts the sequence of SEQ ID NO: 13.

FIG. 17 depicts the sequence of SEQ ID NO: 14.

FIG. 18 depicts the sequence of SEQ ID NO: 15.

FIG. 19 depicts the sequence of SEQ ID NO: 16.

FIG. 20 depicts the sequence of SEQ ID NO: 17.

FIG. 21 depicts the sequence of SEQ ID NO: 18.

FIG. 22 depicts the sequence of SEQ ID NO: 19.

FIG. 23 depicts the sequence of SEQ ID NO: 20.

FIG. 24 depicts the sequence of SEQ ID NO: 21.

FIG. 25 depicts the sequence of SEQ ID NO: 22.

FIG. 26 depicts the sequence of SEQ ID NO: 23.

FIG. 27 depicts the sequence of SEQ ID NO: 24.

FIG. 28 depicts the sequence of SEQ ID NO: 25.

FIG. 29 depicts the sequence of SEQ ID NO: 26.

FIG. 30 depicts the sequence of SEQ ID NO: 27.

FIG. 31 depicts the sequence of SEQ ID NO: 28.

FIG. 32 depicts the sequence of SEQ ID NO: 29.

FIG. 33 depicts the sequence of SEQ ID NO: 30.

FIG. 34 depicts the sequence of SEQ ID NO: 31.

FIG. 35 depicts the sequence of SEQ ID NO: 32.

FIG. 36 depicts the sequence of SEQ ID NO: 33.

FIG. 37 depicts the sequence of SEQ ID NO: 34.

FIG. 38 depicts the sequence of SEQ ID NO: 35.

FIG. 39 depicts the sequence of SEQ ID NO: 36.

FIG. 40 depicts the sequence of SEQ ID NO: 37.

FIG. 41 depicts the sequence of SEQ ID NO: 38.

FIG. 42 depicts the sequence of SEQ ID NO: 39.

FIG. 43 depicts the sequence of SEQ ID NO: 40.

FIG. 44 depicts the sequence of SEQ ID NO: 41.

FIG. 45 depicts the sequence of SEQ ID NO: 42.

FIG. 46 depicts the sequence of SEQ ID NO: 43.

FIG. 47 depicts the sequence of SEQ ID NO: 44.

Description of the Preferred Embodiments

Nucleic Acid Molecules of the Invention

The invention relates to the regulation of cell differentiation and proliferation by administration of nucleic acid molecules encoding parathyroid hormone (PTH), parathyroid hormone related protein (PTHrP), or a fragment or analog thereof. Particular nucleic acid molecules which can be used include those which encode the following peptides:

hPTH (1-84), encoded by nucleotides 1-252 of the nucleic acid molecule of SEQ ID NO: 1 (Kimura, T. *et al.*, *BBRC* 11:493 (1983); Fairwell, T. *et al.*, *Biochemistry* 22:691 (1983)).

hPTH (1-31), encoded by nucleotides 1-93 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (1-34), encoded by nucleotides 1-102 of the nucleic acid molecule of SEQ ID NO: 1.

HPTH (1-36), encoded by nucleotides 1-108 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (1-38), encoded by nucleotides 1-114 of the nucleic acid molecule of SEQ ID NO:1 (Heech, R. D. *et al.*, *Horm. Metab. Res.* 16:556 (1984)).

hPTH (1-44), encoded by nucleotides 1-132 of the nucleic acid molecule of SEQ ID NO:1 (Kimura T. *et al.*, *Biopolymers* 20:1823 (1981)).

hPTH (5-36), encoded by nucleotides 13-108 of the nucleic acid molecule of SEQ ID NO:1.

HPTH (7-34), encoded by nucleotides 19-102 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (13-34), encoded by nucleotides 40-102 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (28-48), encoded by nucleotides 82-144 of the nucleic acid molecule of SEQ ID NO: 1 (Rosenblatt, M. *et al.*, *Biochemistry* 16:2811 (1977)).

HPTH (7-84), encoded by nucleotides 19-252 of the nucleic acid molecule of SEQ ID NO: 1.

hPTH (53-84), encoded by nucleotides 157-252 of the nucleic acid molecule of SEQ ID NO:1 (Rosenblatt, M. *et al.*, *Endocrinology* 103:976 (1978)).

hPTH (64-84), encoded by nucleotides 190-252 of the nucleic acid molecule of SEQ ID NO:1.

hPTH (70-84), encoded by nucleotides 208-252 of the nucleic acid molecule of SEQ ID NO:1.

[Tyr^1]-hPTH (1-34), encoded by nucleotides 1-102 of the nucleic acid molecule of SEQ ID NO: 1, wherein the adenosine at position 2 is mutated to a cytosine.

[Tyr^{27}]-hPTH (27-48), encoded by nucleotides 79-144 of the nucleic acid molecule of SEQ ID NO: 1, wherein the adenosine at position 79 and the guanosine at position 81 are both mutated to a thymidine.

[Tyr^{63}]-hPTH (63-84), encoded by nucleotides 187-252 of the nucleic acid molecule of SEQ ID NO: 1, wherein the cytosine at position 187 is mutated to a thymidine.

[Tyr^{69}]-hPTH (69-84), encoded by nucleotides 205-255 of the nucleic acid molecule of SEQ ID NO: 1, wherein the guanosine at position 205 is mutated to a thymidine, and the guanosine at position 207 is mutated to either a thymidine or a cytosine.

PTH, Bovine (bPTH) (1-84), encoded by nucleotides 1-252 of the nucleic acid molecule of SEQ ID NO: 2.

bPTH (1-34), encoded by nucleotides 1-102 of the nucleic acid molecule of SEQ ID NO:2 (Tregear, G. W. *et al., Biochemistry* 16:2817 (1977)).

bPTH (3-34), encoded by nucleotides 7-102 of the nucleic acid molecule of SEQ ID NO: 2 (Lowrik, C. *et al., Cell Calcium* 6:311 (1985)).

PTHrP (1-31), encoded by nucleotides 1-93 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (1-40), encoded by nucleotides 1-120 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (5-36), encoded by nucleotides 13-108 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (7-34), encoded by nucleotides 19-102 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (7-139), encoded by nucleotides 19-417 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (7-141), encoded by nucleotides 19-423 of the nucleic acid molecule of SEQ ID NO: 3.

PTHrP (7-173), encoded by nucleotides 19-519 of the nucleic acid molecule of SEQ ID NO: 3.

Rat PTH (rPTH) (1-84), encoded by nucleotides 1-252 of the nucleic acid molecule of SEQ ID NO: 4 (Heinrich, G. *et al., J. Biol. Chem.* 25:3320 (1984)).

In addition, nucleic acid molecules which encode the peptides and peptide derivatives disclosed in the following documents can also be used: U.S. Pat. Nos. 4,086,196, 4,423,037, 4,771,124, 4,833,125, 4,968,669, 5,001,223, 5,087,562, 5,093,233, 5,116,952, 5,149,779, 5,171,670, 5,229,489, 5,317,010, 5,382,658, 5,393,869, 5,434,246, 5,527,772, 5,589,452, 5,807,823, 5,821,255, 5,840,690, 5,977,070, 6,025,467, 6,051,868, and 6,066,618; WO94/02510, WO00/23594, and WO00/31137; and EP 477,885.

A typical design for constructing the PTH (7-34), (7-84), (7-141), and PTHrP (7-34), (7-139), and (7-173) fragment cDNAs is to place a ATG start codon upstream of the initial peptide codon of the individual fragments and to introduce a stop codon downstream of the final peptide codon of the individual fragments. Also, an endogenous peptide cleavage site will be introduces between the ATG start codon and the initial peptide codon of the individual fragments to avoid unwanted amino acids being introduced into the constructs.

When selecting a candidate nucleic acid molecule for a method of this invention, a preferred first step is to choose a nucleic acid molecule encoding a peptide which includes a fragment which has at least 10%, and more preferably 50% or greater, sequence identity with an 8 or greater amino acid long fragment within the amino terminal 34 amino acid region of hPTH or hPTHrP. The term "sequence identity" refers to a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. (See, *e.g.*: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer*

Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H. & Lipton, D., *SIAM J Applied Math 48*:1073 (1988)). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H. & Lipton, D., *SIAM J Applied Math 48*:1073 (1988). Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., *et al., Nucleic Acids Research 12(i)*:387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al., J Molec Biol 215*:403 (1990)).

Therefore, as used herein, the term "identity" represents a comparison between a test and reference sequence. More specifically, reference test sequence is defined as any test sequence that is 10% or more identical to a reference sequence. As used herein, the term at least 10% identical to refers to percent identities from 10 to 99.99 relative to the reference sequence. Identity at a level of 10% or more is indicative of the fact that, assuming for exemplification purposes a test and reference sequence length of 100 amino acids, that no more than 90% (i.e., 90 out of 100) of the amino acids in the test sequence differ from that of the reference sequence. Such differences may be represented as point mutations randomly distributed over the entire length of the nucleotide or amino acid sequence of the invention or they may be clustered in one or more locations of varying length up to the maximum allowable amino acid difference. Differences are defined as nucleotide or amino acid substitutions, or deletions.

Because of the high degree of homology among human PTH and PTH of other species, PTH peptides encoded by nucleic acids from non-human as well as human sources can be used. Similarly, human PTHrP (1-139), (1-141) and (1-173) have a high degree of homology with PTHrP of other species; therefore, nucleic acids from non-human as well as human sources can be used in the methods of the invention involving PTHrP.

Candidate nucleic acid molecules may be tested for suitability as inhibitors of cell proliferation and enhancers of differentiation using cultured human keratinocytes, similar to the method for testing peptides described in U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618. Briefly, those nucleic acid molecules encoding peptides which inhibit proliferation and induce differentiation in cultured keratinocytes are those potentially useful as therapeutic agents in treating disorders, e.g., psoriasis and cancer, where suppression of cell proliferation is desired. Candidate nucleic acid molecules may be tested for suitability as enhancers of cell proliferation using cultured human keratinocytes or *in vivo* mouse model. Those peptides encoded by the nucleic acid molecules which block the effect of agonist peptides or $1,25(OH)_2D_3$ on cultured keratinocyte proliferation are those potentially useful as therapeutic agents in treating disorders, e.g., wounds, burns, or skin ulcerations, where maintenance or stimulating of cell proliferation is desired.

Candidate nucleic acid molecules may be tested for their ability to enhance wound healing by carrying out a skin punch biopsy test, as described in U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618.

Candidate peptides may be tested for suitability as stimulators of hair growth using an in vitro hair growth assay, as described in U.S. Pat. Nos. 5,527,772, 5,840,690 and 6,066,618. Those peptides encoded by the nucleic acid molecules which stimulate hair growth in vitro are those potentially useful for the stimulation of hair growth in vivo, e.g., for the stimulation or maintenance of hair growth during or following chemotherapy or to treat a form of alopecia, e.g., male and female pattern baldness.

Alternatively, *in vivo* assays may be carried out as described herein and similar to those described in Schilli, M.B. *et al., J. Invest. Dermatol. 108*:928-932 (1997); Holick, M.F., *et al., Proc. Natl. Acad. Sci. 91*:8014-8016 (1994); Paus, R. and Cotsarelis, G., *N. Engl. J. Med. 341*: 491-497 (1999); Paus, R., *et al. Laboratory Invest. 60*: 365-369 (1989) and U.S. Pat. App. No. 60/213,247.

Care should be taken when determining the correct nucleic acid molecule for use in the invention. Experiments have shown that when normal cultured human keratinocytes are transfected with plasmids containing PTHrP (1-141) or PTHrP (1-173) an unexpected enhancement of cell growth is seen, as measured by ^{3}H-thymidine incorporation into epidermal DNA (FIG. 1). These results are attributed to proteolysis of the full-length peptide. For this reason, all candidate nucleic acid molecules should be tested for the expected activity before use.

Gene Therapy

In this preferred embodiment of the invention, a nucleic acid molecule encoding a peptide with desired activity is incorporated into a polynucleotide construct suitable for introducing the nucleic acid molecule into cells of the animal to be treated, to form a transfection vector. The transfection vector is then introduced into selected target tissues of the cells of the animal *in vivo* using any of a variety of methods known to those skilled in the art. Alternatively, naked DNA may be transfected into the cells, with or without cationic lipids.

Techniques for the construction of transfection vectors containing inserts of desired nucleic acid sequences are well-known in the art, and are generally described in "Working Toward Human Gene Therapy," Chapter 28 in *Recombinant DNA, 2nd Ed.*, Watson, J.D. *et al.* (eds.), Scientific American Books: New York (1992), pp. 567-581, or Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York (1989).

Gene therapy approaches that may be used to deliver a nucleic acid molecule include injection of plasmid DNA (Horton, H.M., *et al.*, *Proc. Natl. Acad. Sci. USA 96(4)*:1553-1558 (1999)); transduction using adenoviral vectors (Waugh, J.M., *et al.*, *Proc. Natl. Acad. Sci. USA 96(3)*:1065-1070 (1999)); transduction using retrovial vectors (Axelrod, J.H., *et al.*, *Proc. Natl. Acad. Sci. USA 87*:5173-5177 (1990); Drumm, M.L., *et al.*, *Cell 62*:1227-1233 (1990); Krueger, G.G., *et al.*, *J. Invest. Dermatol. 112*:233-239 (1999); Palmer, T.D., *et al.*, *Blood 73*:438-445 (1989); and Rosenberg, S.A., *et al.*, *N. Eng. J. Med. 323*:570-578 (1990)); and gene transfer using liposomes (Mason, C.A.E., *et al.*, *Nature Medicine 5(2)*:176-182 (1999)). In addition, general methods for construction of gene therapy vectors and the introduction of such vectors into a mammal for therapeutic purposes may be obtained in the above-referenced publications, the disclosures of which are specifically incorporated herein by reference in their entirety. In one such general method, vectors containing nucleic acid sequences of the present invention are directly introduced into the cells or tissues of the mammal to be treated, preferably by topical application. Such an approach is generally referred to as "*in vivo*" gene therapy.

Alternatively, cells or tissues may be removed from the mammal to be treated and placed into culture according to methods that are well-known to one of ordinary skill in the art. Transfection vectors or naked DNA containing the genes for desired peptides may then be introduced into these cells or tissues by any of the methods described generally above for introducing isolated polynucleotides into a cell or tissue. After a sufficient amount of time to allow incorporation of the inserted DNA, the cells or tissues may then be re-inserted into the mammal to be treated. Since introduction of the nucleic acid molecule encoding the peptide is performed outside of the body of the mammal, this approach is generally referred to as "*ex vivo*" gene therapy. See U.S. Patent No. 5,399,346. Gene transfer through transfection of cells *ex vivo* can be performed by a variety of methods, including, for example, calcium phosphate precipitation,

diethylaminoethyl dextran, electroporation, lipofection, or viral infection. Such methods are well known in the art (see, for example, Sambrook *et al.*).

For both *in vivo* and *ex vivo* gene therapy, the nucleic acid molecule encoding the desired peptide of the invention may be operatively linked to a regulatory DNA sequence, or "promoter," to form a genetic construct as described above. This construct, containing both the promoter and the nucleic acid molecule encoding the peptide, may be subcloned into a suitable vector such as a plasmid, adenovirus vector, retrovirus vector, or the like, and introduced into the animal to be treated in an in vivo gene therapy approach, or into the cells or tissues of the mammal in an ex vivo approach.

Alternatively, the nucleic acid molecule of the invention may be operatively linked to a heterologous regulatory DNA sequence, or promoter, to form a genetic construct as described above. The heterologous regulatory sequence may be tissue specific. The vector containing the genetic construct is then directly introduced into the animal to be treated or into the cells or tissues of the animal, as described.

The term "operably linked", as used herein, denotes a relationship between a regulatory region (typically a promoter element, but may include an enhancer element) and the gene, whereby the transcription of the gene is under the control of the regulatory region.

The term "heterologous" means a DNA sequence not found in the native genome. That is, two nucleic acid elements are said to be "heterologous" if the elements are derived from two different genes, or alternatively, two different species. Thus, "heterologous DNA regulatory sequence" indicates that the regulatory sequence is not naturally ligated to the nucleic acid molecule selected for use in the invention.

The term "promoter" is used according to its art-recognized meaning. It is intended to mean the DNA region, usually upstream to the coding sequence of a gene, which binds RNA polymerase and directs the enzyme to the correct transcriptional start site.

In general, a promoter may be functional in a variety of tissue types and in several different species of organisms, or its function may be restricted to a particular species and/or a particular tissue. Further, a promoter may be constitutively active, or it may be selectively activated by certain substances (e.g., a tissue-specific factor), under certain conditions (e.g., in the presence of an enhancer element, if present, in the genetic construct containing the promoter), or during certain developmental stages of the organism (e.g., active in fetus, silent in adult).

Promoters useful in the practice of the present invention are preferably "tissue-specific"--that is, they are capable of driving transcription of a gene in one tissue while remaining largely "silent" in other tissue types. Examples of tissue-specific promoters in the skin are the Keratin promoter (Vassar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 86:8565 (1989)), the POMC promoter (Deen *et al. Mol. Biol. Evol.* 9:483 (1992)), the alpha-actin promoter (Shani, *Mol. Cell. Biol.*, 6:2624 (1986)), the elastase-q promoter (Swift *et al., Cell* 28:639 (1984)), the tyrosine hydroxylase promoter (Kim, L. S., *et al., J. Biol. Chem 268*:15689 (1993); Kaneda, N., *et al., Neuron 6*:583 (1991)), the dopamine beta-hydroxylase promoter (Mercer E. H., *et al., Neuron* 7:703 (1991); Hcyle, G. W., *et al., J. Neurosci. 14*:2455 (1994)), the tryptophan hydroxylase promoter (Boularand, S., *et al., J. Biol. Chem 270*:3757 (1995); Stoll, J. and Goldman, D., *J. Neurosci. Res. 28*:457 (1991)) and the parathyroid hormone-related peptide promoter (Campos, R. V., *et al., Mol. Rnfovtinol. 6*:1642). For additional examples of tissue-specific promoters, see U.S. Patent Nos. 5,834,306 and 5,416,027, and references cited therein.

In addition to a promoter, the genetic construct may also contain other genetic control elements, such as enhancers, repressible sequences, and silencers, which may be used to regulate replication of the vector in the target cell. The only requirement is that the genetic element be activated, derepressed, enhanced, or otherwise genetically regulated by factors in the host cell and, with respect to methods of treatment, not in the non-target cell.

An "element," when used in the context of nucleic acid constructs, refers to a region of the construct or a nucleic acid fragment having a defined function.

For example, an enhancer element, as used herein, is a region of DNA that, when associated with inserted nucleic acid molecule, operably linked to a promoter, enhances the transcription of that gene.

The term "enhancer" is used according to its art-recognized meaning. It is intended to mean a sequence found in eukaryotes which can increase transcription from a gene when located (in either orientation) up to several kilobases from the gene being studied. These sequences usually act as enhancers when on the 5′ side (upstream) of the gene in question. However, some enhancers are active when placed on the 3′ side (downstream) of the gene. In some cases, enhancer elements can activate transcription from a gene with no (known) promoter.

Preferred enhancers include the DF3 breast cancer-specific enhancer and enhancers from viruses and the steroid receptor family. Other preferred transcriptional regulatory sequences include NF1, SP1, AP1, and FOS/JUN.

Any of a variety of methods known to those skilled in the art may be used to introduce transfection vectors of the present invention into selected target tissue cells. Such methods include, for example, viral-mediated gene transfer using retroviruses, adeno-associated virus (AAV), herpes virus, vaccinia virus, or RNA viruses (*e.g.,* Grunhaus and Horowitz, *Semin. Virol. 3*:237-252 (1992); Herz and Gerard, *Proc. Nat. Acad. Sci. USA 90*:2812-2816 (1993); and Rosenfeld *et al.*, *Cell 68*:143-155 (1992)); liposome-mediated gene transfer (Morishita *et al.*, *J. Clin. Invest. 91*:2580 (1993); Felgner *et al.*, U.S. Patent Nos. 5,703,055 (1997) and 5,858,784 (1999)); injection of naked DNA directly into a target tissue (*e.g.*, Felgner *et al.*, U.S. Patent No. 5,589,466 (1996); Wolff *et al.*, U.S. Patent No. 5,693,622 (1997)); and receptor-mediated gene transfer (Wu and Wu, *Biochemistry 27*:887-892 (1988); Wagner *et al.*, *PNAS USA 87*:3410-3414 (1990); Curiel *et al.*, U.S. Patent 5,547,932 (1996); and Beug *et al.*, U.S. Patent No. 5,354,844 (1994)).

In any of these methods, where a vector may be targeted to selectively transfect a specific population of cells, it will be understood that in addition to local administration (such as may be achieved by injection into the target tissue), the vector may be administered systemically (e.g., intravenously) in a biologically-compatible solution or pharmaceutically acceptable delivery vehicle. Vector constructs administered in this way may selectively infect the target tissue. According to the present invention, the presence of a target tissue-specific promoter on the construct provides an independent means of restricting expression of the therapeutic gene.

Nucleic acid molecules encoding peptides which block antiproliferative compounds can also be useful in conjunction with chemotherapeutic agents in the treatment of skin cancer; many chemotherapeutic agents are effective only against dividing cells, and the blocking peptides can have the effect of inducing division of otherwise dormant cells, rendering them vulnerable to the chemotherapy. Nucleic acids encoding blocking peptides can also be useful in promoting growth of new cells, e.g., skin cells, in topical skin creams. Differentiation-inducing peptides can be used as immunostimulants, by inducing maturation of monocytes and lymphocytes bearing PTH receptors, while blocking peptides can be used to inhibit lymphocyte maturation, and thus can be used to treat conditions, e.g., autoimmune diseases such as juvenile diabetes, rheumatoid arthritis, and allograft rejection, where mature lymphocytes are a causative agent.

The nucleic acid molecules of the invention can be admixed with a pharmacologically inert topical carrier such as one comprising a gel, an ointment or a cream, including such carriers as water, glycerol, alcohol, propylene glycol, fatty alcohol, triglycerides, fatty acid ester or mineral oils. Other possible carriers are liquid petrolatum, isopropylpalmitate, polyethylene glycol ethanol 95%, polyoxyethylene monolaurate 5% in water, sodium lauryl sulfate 5% in water, and the like. Materials such as antioxidants, humectants, viscosity stabilizers and the like may be added, if necessary. Nucleic acid molecules can be incorporated into liposomes using methods outlined in U.S. Pat. 5,260,065.

The nucleic acid molecules can be incorporated into a collagenous biocompatable matrix similar to the methods utilized in Fang *et al., Proc. Nat. Acad. Sci. U.S.A.* 93:5753 (1996) and U.S. Pat. 5,962,427. The types of matrices that may be used in the practice of the invention is virtually limitless and may include both biological and synthetic matrices. The matrices may be biodegradable or non-biodegradable. The matrices may take the form of sponges, implants, tubes, telfa pads, band-aids, bandages, pads, lyophylized components, gels, patches, powders or nanoparicles. Particular examples of such matrices include porous or collagenous materials (*e.g.* type II collagen), hydroxyapatite, bioglass, aluminates, bioceramic materials, purified proteins or extracellular matrix compositions as well as metals such as titanium.

The nucleic acid molecules can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those of therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as hydrohalic acids, e.g, hydrochloric acid, sulfuric acid, or phsophoric acid.

Dosage will be dependent upon the age, health, and weight of the recipient; kind of concurrent treatment, if any; frequency of treatment; and the nature of the effect desired. Generally, daily dosage may be 0.001 to 500 μg/kg. The topical dosage may be from 0.01 to 100 $\mu g/cm^2$. The liposomal gel, ointment or cream formulations may be applied by one or more applications per day.

The invention also relates to compositions comprising a nucleic acid molecule of the invention, an active vitamin D compound and a pharmaceutical carrier, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when

expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair growth. A large number of active vitamin D compounds are known which can be used in the practice of the present invention. See U.S. Patent Nos. 5,457,217, 5,414,098, 5,384,313, 5,373,004, 5,371,249, 5,430,196, 5,260,290, 5,393,749, 5,395,830, 5,250,523, 5,247,104, 5,397,775, 5,194,431, 5,281,731, 5,254,538, 5,232,836, 5,185,150, 5,321,018, 5,086,191, 5,036,061, 5,030,772, 5,246,925, 4,973,584, 5,354,744, 4,927,815, 4,857,518, 4,851,401, 4,851,400, 4,847,012, 4,755,329, 4,940,700, 4,619,920, 4,594,192, 4,588,716, 4,564,474, 4,552,698, 4,588,528, 4,719,204, 4,719,205, 4,689,180, 4,505,906, 4,769,181, 4,502,991, 4,481,198, 4,448,726, 4,448,721, 4,428,946, 4,411,833, 4,367,177, 4,336,193, 4,360,472, 4,360,471, 4,307,231, 4,307,025, 4,358,406, 4,305,880, 4,279,826, and 4,248,791. A preferred active vitamin D compound is calcipotriene. In this embodiment, any conventional liposome may be used including the liposomes described in U.S. Pat. Nos. 4,235,871, 4,241,046, 4,247,411, 4,356,167, 4,377,567, 4,544,545, 4,551,288, 4,610,868, 4,731,210, 4,744,989, 4,772,471, 4,897,308, 4,917,951, 5,021,200, 5,032,457, and 5,260,065.

The invention relates as well to a method of inhibiting proliferation or enhancing differentiation of a skin or hair cell of a mammal, comprising administering to the mammal in need thereof a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule of the invention and an active vitamin D compound, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth. In this embodiment, the nucleic acid molecule encoding the peptide

and the active vitamin D compound may be administered as part of single or separate pharmaceutical compositions. Either one or both of the nucleic acid molecules and active vitamin D compound may be administered topically or parenterally. In a preferred embodiment, the nucleic acid molecule is administered first followed by the active vitamin D compound.

The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in clinical therapy and which are obvious to those skilled in the art are within the spirit and scope of the invention.

Example 1

Mini-gene construction

PTHrP gene: PTHrP gene expresses three isoform peptides: PTHrP 1-139, PTHrP1-141 and PTHrP 1-173. The gene splicing happens between exon 4 to exon 6. The 5'-flanking regions share common nucleotide sequences, including precursor peptide. PTHrP mini-genes were made based on the nucleotide sequences of the human PTHrP/PLP gene, (Yasuda *et al. J. Biol. Chem.* 264:7720 (1989)) by using the PCR technique. The interested gene fragments were constructed into pCR3.1eukaryotic expression vector. The forward primer for PTHrP (1-139), PTHrP (1-141), PTHrP (1-173) and PTHrP (1-34) is 5'-AGCGGAGACGATGCAGCGGAGA-3' (SEQ ID NO: 26), reverse primer for PTHrP (1-139) is 5'-AAGGGAGGCAGCTGAGACG-3' (SEQ ID NO: 27), for PTHrP (1-141) is 5'-GTCCTTGGAAGGTCTCTGCTG-3' (SEQ ID NO: 28), for PTHrP (1-173) is 5'-TTCTAGTGCCACTGCCCATTG-3' (SEQ ID NO:29) and for PTHrP (1-34) is 5'-CTACTAAGCTGTGTGGATTTCTGCGAT-3' (SEQ ID NO:

30). PCR was performed at 94°C for 3 min initial denaturing, then followed by denaturing for 30 seconds at 94°C, annealing for 30 seconds at 60°C and extension for 1 min at 72°C, total 30 cycles, additional extension for 10 min at 72°C.

Adenovirus construction of PTHrP

The corresponding mature and fragment forms of PTH or PTHrP cDNAs (FIG. 2) can be subcloned into the adenovirus expression vector, pACCMV.pLpA (FIG. 3). Once the PTH and PTHrP inserts are subcloned and purified they are co-transfected with pJM17 in 293 cells, which contains essential elements of the adenovirus genome to replicate and produce recombinant virions. The virions isolated for the co-transfected 293 cells are infectious but don't have the capacity to replicated in other cell types except 293 cells with the pJM17 vector. The purified pACCMV.pLpA. PTHrP virion particles can then be used for gene transfer of the various PTHrPs cDNAs driven by the CMV promoter in culture and animals (Tomas C. Berker, *et al.* Methods of Cell Biology, Use of Recombinant Adenovirus for Metabolic Engineering of Mammalian Cells, Vol. 43, Chp 8; pg. 161-187, Academic Press Inc., San Diego, CA., USA. 1994).

Transfection

Keratinocytes were maintained in MCDB-153 medium. Cells in 24 well dishes at 50%-60% confluence were transfected with 1 μg/ml of PTHrP cDNA which was constructed into pCR3.1 vector (INVITROGEN, San Diego, CA., USA), empty vector as a control. For each transfection, 0.5 micrograms of DNA and 3 microliters of LIPOFECTAMINE were diluted in 50 microliters of serum free media, respectively, and then combined for a DNA/ Liposome

complexing incubation for 15 minute at room temperature. DNA/ Liposome complex was then incubated on the cells for 3 hours. After 3 hour of transfection, fresh media was added and cells were incubated for 21 hours.

^3H - Thymidine Incorporation

^3H-thymidine incorporation into DNA was used as an index of cell proliferation as described previously (Smith E.L. *et al. J. Invert. Dermatology* 86:709 (1986), Holick *et al. Proc. Nat. Acad. Sci. U.S.A.* 91:8014 (1994)). Twenty-four hours post transfection the medium was replaced with 0.5 ml of fresh basal medium containing [methyl-^3H]thymidine (New England Nuclear, Boston, MA) and incubated for 3 h at 37° C. ^3H-Thymidine incorporation into DNA was stopped by placing the 24-well plates on ice. Unincorporated ^3H-thymidine was then removed and the cells were washed three times with ice-cold phosphate-buffered saline. DNA labeled with ^3H-thymidine and other macromolecules were first precipitated with ice-cold 5% perchloric acid for 20 min and then extracted with 0.5 ml of 5% perchloric acid at 70 °C for 20 min. The radioactivity in the extracts was determined by a liquid scintillation counter. The results were expressed as percent of control.

Experiments have shown that when normal cultured human keratinocytes are transfected with plasmids containing PTHrP (1-141) or PTHrP (1-173) an unexpected enhancement of cell growth is seen, as measured by ^3H-thymidine incorporation into epidermal DNA (FIG. 1). These results may be due to proteolysis of the full length peptide.

Having now fully described this invention, it will be understood by those of ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any embodiment thereof. All patents, patent applications and publications cited herein are fully incorporated by

reference herein in their entirety.

What Is Claimed Is:

1. A method of inhibiting proliferation or enhancing differentiation of a mammalian skin or hair cell, said method comprising administering to the mammalian skin or hair cell in need of inhibited proliferation or enhanced differentiation with a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth.

2. The method of claim 1, wherein said nucleic acid molecule is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

3. The method of claim 2, wherein said carrier is a liposome.

4. The method of claim 1, wherein said nucleic acid molecule is contained within a porous biocompatable matrix.

5. The method of claim 1, wherein said peptide encoded by the nucleic acid molecule is PTH (1-34) (SEQ ID NO: 18), PTHrP (1-34) (SEQ ID NO: 31), PTH (1-84) (SEQ ID NO: 15), PTHrP (1-141) (SEQ ID NO: 32), PTHrP (1-139) (SEQ ID NO: 33) or PTHrP (1-173) (SEQ ID NO: 34).

6. The method of claim 1, wherein said nucleic acid molecule is one of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, or a fragment thereof.

7. The method of claim 1, wherein said nucleic acid molecule is administered topically to the mammalian skin or hair cells.

8. The method of claim 1, wherein said method is a method of inhibiting a hyperproliferative skin disorder.

9. The method of claim 8, wherein said hyperproliferative skin disorder is psoriasis, ichthyosis, eczema, acne, actinic keratosis, or skin cancer.

10. The method of claim 1, wherein said method is a method of inhibiting hair growth or preventing hair regrowth.

11. The method of claim 1, wherein said peptide encoded by the nucleic acid molecule has at least 75% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP.

12. The method of claim 1, further comprising administering to the mammalian hair or skin cell an effective amount of an active vitamin D compound.

13. The method of claim 12, wherein said active vitamin D compound is calcipotriene.

14. The method of claim 12, wherein said active vitamin D compound is 1,25-dihydroxyvitamin D_3.

15. The method of claim 12, wherein said active vitamin D compound is 19-nor-1,25-dihydroxyvitamin D_2.

16. The method of claim 12, wherein said active vitamin D compound is 19-nor-1,25-dihyroxyvitamin D_3.

17. The method of claim 12, wherein said nucleic acid molecule and active vitamin D compound are administered topically or parenterally.

18. The method of claim 1, wherein said nucleic acid molecule is operably linked to a promoter.

19. The method of claim 1, wherein said nucleic acid molecule is contained by a plasmid.

20. The method of claim 1, wherein said nucleic acid molecule is contained by a viral vector.

21. A method of inhibiting proliferation or enhancing differentiation of a skin or hair cell of a mammal, said method comprising administering to the mammal in need thereof a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule and an active vitamin D compound, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth.

22. The method of claim 21, wherein said nucleic acid molecule and said active vitamin D compound are administered as part of a single pharmaceutical composition.

23. The method of claim 21, wherein said nucleic acid molecule and said active vitamin D compound are administered as part of separate pharmaceutical compositions.

24. The method of claim 21, wherein said nucleic acid molecule is administered parentally.

25. The method of claim 21, wherein said active vitamin D compound is administered topically.

26. The method of claim 21, wherein said active vitamin D compound is administered orally.

27. The method of claim 21, wherein said nucleic acid molecule is encapsulated within a liposome.

28. The method of claim 21, wherein said nucleic acid molecule is contained within a porous biocompatable matrix.

29. A method of inducing proliferation of a mammalian skin or hair cell, said method comprising administering to the mammalian skin or hair cell in need of proliferation with a proliferation-inducing amount of a nucleic acid molecule, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of blocking the inhibition of proliferation or stimulation of differentiation in vitro

of cultured human keratinocytes by PTH (1-34), $1,25(OH)_2D_3$ or PTHrP (1-34), or *in vivo* in mouse skin by stimulating skin cell proliferation or accelerating hair cycle progression or stimulating hair cell growth.

30. The method of claim 29, wherein said nucleic acid molecule is administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

31. The method of claim 30, wherein said carrier is a liposome.

32. The method of claim 29, wherein said nucleic acid molecule is contained within a porous biocompatable matrix.

33. The method of claim 29, which is a method of stimulating skin cell growth, rejuvenating aged skin, preventing skin wrinkles, treating skin wrinkles, enhancing wound healing, stimulating hair growth, maintaining hair growth, treating or preventing female or male pattern baldness, or treating chemotherapy induced alopecia.

34. The method of claim 29, which is a method of stimulating epidermal cell growth or hair follicle cell growth.

35. The method of claim 29, wherein said peptide encoded by the nucleic acid molecule is PTH (7-34) (SEQ ID NO: 35), PTHrP (7-34) (SEQ ID NO: 36), PTH (5-36) (SEQ ID NO: 37), PTHrP (5-36) (SEQ ID NO: 38), PTH (5-34) (SEQ ID NO: 39), PTHrP (5-34) (SEQ ID NO: 40), PTH (7-84) (SEQ ID NO: 12), PTHrP (7-139) (SEQ ID NO: 41), PTHrP (4-141) (SEQ ID NO: 42), or PTHrP (7-173) (SEQ ID NO: 43)

36. The method of claim 1, wherein said nucleic acid molecule is one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, or a fragment thereof.

37. A composition comprising a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule encapsulated within a liposome, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth.

38. The method of claim 37, wherein said nucleic acid molecule is contained within a porous biocompatable matrix.

39. A composition comprising a proliferation-inducing amount of a nucleic acid molecule encapsulated within a liposome, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of blocking the inhibition of proliferation or stimulation of differentiation in vitro of cultured human keratinocytes by PTH (1-34), $1,25(OH)_2D_3$ or PTHrP (1-34), or *in vivo* in mouse skin by stimulating skin cell proliferation or accelerating hair cycle progression or stimulating hair cell growth.

40. A composition comprising a proliferation-inhibiting or differentiation-enhancing amount of a nucleic acid molecule and an active vitamin D compound, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity

with the 34 amino acid N-terminal region of hPTH or hPTHrP, and, when expressed, is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or *in vivo* in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth.

41. The composition of claim 40, wherein at least one of said nucleic acid molecules or active vitamin D compound is encapsulated by liposomes.

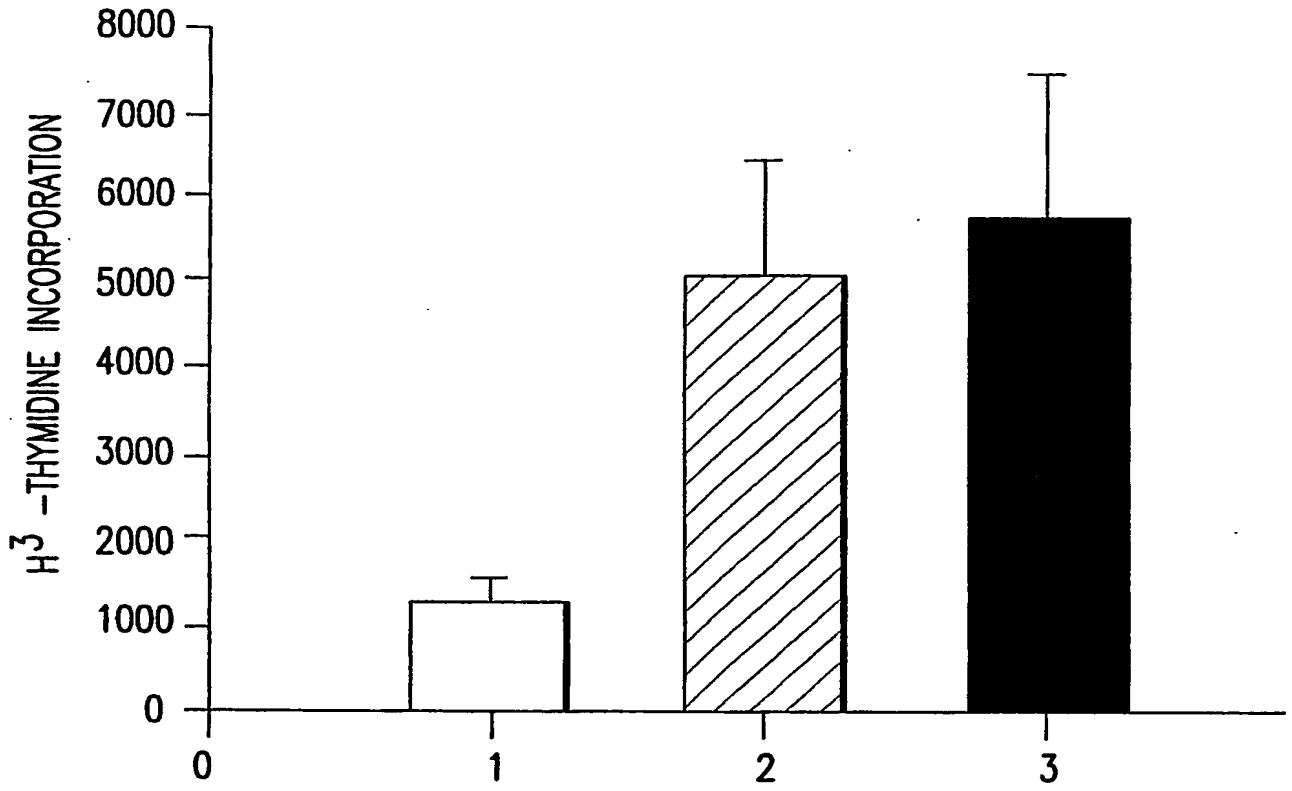
8000
7000
6000
5000
4000
3000
2000
1000
0
H^3 -THYMIDINE INCORPORATION
0
1
2
3

FIG. 1

PTHrP cDNA STRUCTURE

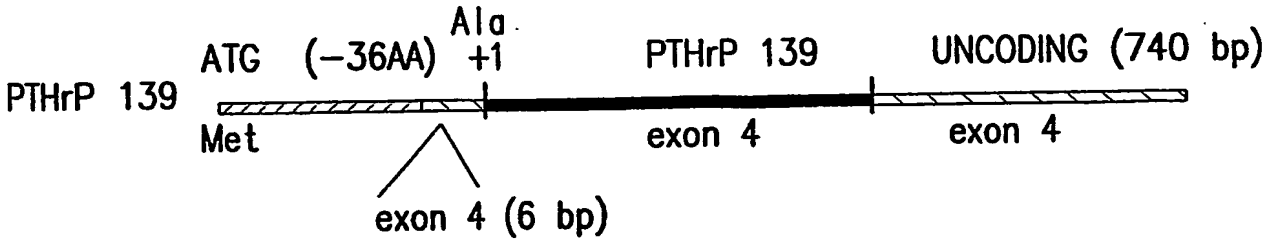

FIG.2A

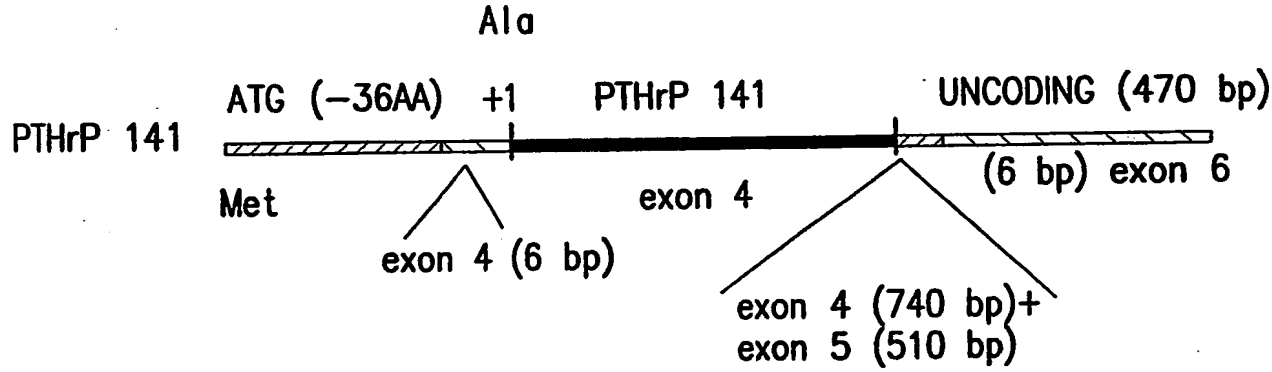

FIG.2B

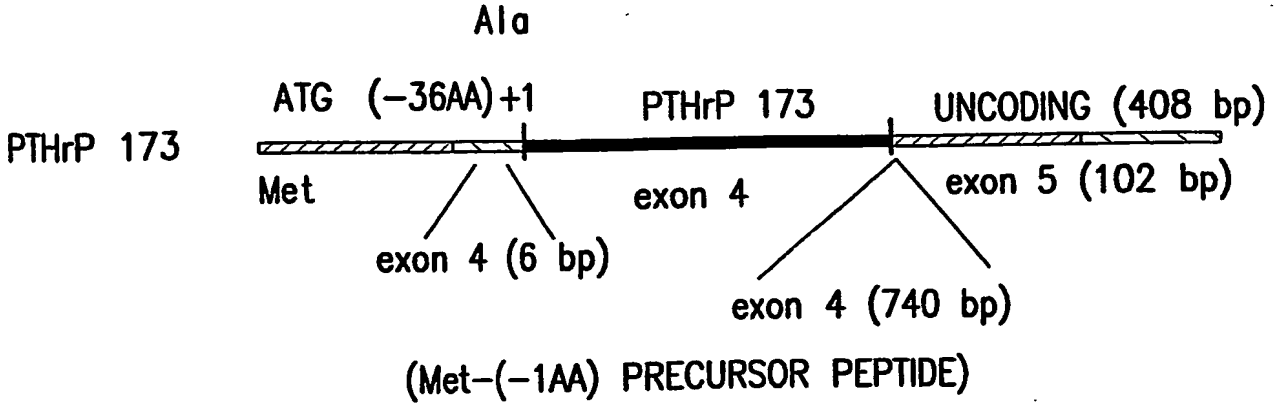

FIG.2C

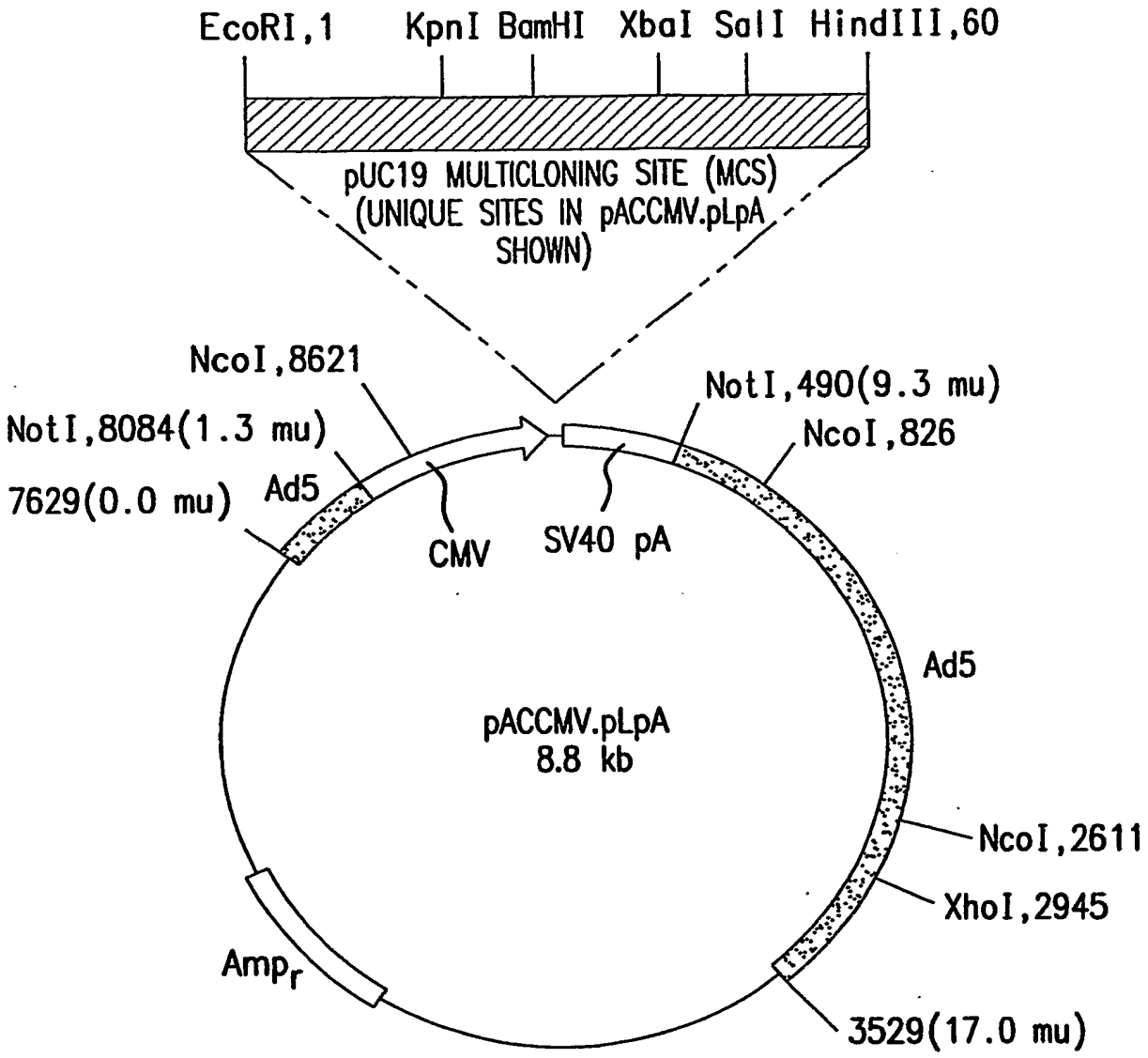
EcoRI,1
KpnI BamHI XbaI SalI HindIII,60
pUC19 MULTICLONING SITE (MCS)
(UNIQUE SITES IN pACCMV.pLpA
SHOWN)
NcoI,8621
NotI,8084(1.3 mu)
7629(0.0 mu)
Ad5
CMV
SV40 pA
NotI,490(9.3 mu)
NcoI,826
Ad5
pACCMV.pLpA
8.8 kb
NcoI,2611
XhoI,2945
3529(17.0 mu)
Amp$_r$

FIG.3

SEQ ID NO: 1 - Human Parathyroid Hormone Coding Sequence (hPTH)
TCTGTGAGTGAAATACAGCTTATGCATAACCTGGGAAAACATCTGAACTC
GATGGAGAGAGTAGAATGGCTGCGTAAGAAGCTGCAGGATGTGCACAATT
TTGTTGCCCTTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTTCCCAGAGG
CCCCGAAAAAAGGAAGACAATGTCTTGGTTGAGAGCCATGAAAAAAGTCT
TGGAGAGGCAGACAAAGCTGATGTGAATGTATTAACTAAAGCTAAATCCC
AGTGA

FIG.4

SEQ ID NO: 2 - Bovine Parathyroid Hormone Coding Sequence (bPTH)
GCTGTGAGTGAAATACAGTTTATGCATAACCTGGGCAAACATCTGAGCTC
CATGGAAAGAGTGGAATGGCTGCGGAAAAAGCTACAGGATGTGCACAACT
TTGTTGCCCTTGGAGCTTCTATAGCTTACAGAGATGGTAGTTCCCAGAGA
CCTCGAAAAAAGGAAGACAATGTCCTGGTTGAGAGCCATCAGAAAAGTCT
TGGAGAAGCAGACAAAGCTGATGTGGATGTATTAATTAAAGCTAAACCCC
AG

FIG.5

SEQ ID NO: 3 - Human Parathyroid Hormone Related Protein Coding Sequence(PTHrP)
GCTGTGTCTGAACATCAGCTCCTCCATGACAAGGGGAAGTCCATCCAAGA
TTTACGGCGACGATTCTTCCTTCACCATCTGATCGCAGAAATCCACACAG
CTGAAATCAGAGCTACCTCGGAGGTGTCCCCTAACTCCAAGCCCTCTCCC
AACACAAAGAACCACCCCGTCCGATTTGGGTCTGATGATGAGGGCAGATA
CCTAACTCAGGAAACTAACAAGGTGGAGACGTACAAAGAGCAGCCGCTCA
AGACACCTGGGAAGAAAAAGAAAGGCAAGCCCGGGAAACGCAAGGAGCAG
GAAAAGAAAAAACGGCGAACTCGCTCTGCCTGGTTAGACTCTGGAGTGAC
TGGGAGTGGGCTAGAAGGGGACCACCTGTCTGACACCTCCACAACGTCGC
TGGAGCTCGATTCACGGAGGCATTGA

FIG.6

SEQ ID NO: 4 - Rat Parathyroid Hormone Coding Sequence (rPTH)
GCTGTCAGTGAAATACAGCTTATGCACAACCTGGGCAAACACCTGGCCTC
TGTGGAGAGGATGCAATGGCTGAGAAAAAAGCTGCAAGATGTACACAATT
TTGTTAGTCTTGGAGTCCAAATGGCTGCCAGAGAAGGCAGTTACCAGAGG
CCCACCAAGAAGGAGGAAAATGTCCTTGTTGATGGCAATTCAAAAAGTCT
TGGCGAGGGGGACAAAGCTGATGTGGATGTATTAGTTAAGGCTAAATCTC
AGTAA

FIG.7

SEQ ID NO:5 - hPTH (1-31)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDV

FIG.8

SEQ ID NO:6 - PTHrP - (1-40)
H_2N-Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Phe-Phe-Leu-His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-Glu-Ile-Arg-Ala-Thr-Ser-OH

FIG.9

SEQ ID NO:7 - PTH, Bovine (bPTH) (84 amino acids)
H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Ser-Ile-Ala-Tyr-Arg-Asp-Gly-Ser-Ser-Gln-Arg-Pro-Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Gln-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Ile-Lys-Ala-Lys-Pro-Gln-OH

FIG10

SEQ ID NO:8 - [Tyr^{63}] -hPTH (63-84)
H_2N-Tyr-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG.11

SEQ ID NO:9 - hPTH (64-84)
H_2N-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG.12

SEQ ID NO:10 - [Tyr^{69}] -hPTH (69-84)
H_2N-Tyr-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG.13

SEQ ID NO:11 - hPTH (70-84)
H_2N-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG.14

SEQ ID NO:12 - hPTH (7-84)
H_2N-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-Ala-Gly-Ser-Gln-Arg-Pro-Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH

FIG.15

SEQ ID NO:13 - hPTHrP (1-31)-
AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIH

FIG.16

SEQ ID NO:14 - hPTH (1-34)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF

FIG.17

SEQ ID NO:15 - hPTH (84 amino acids)
H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-Ala-Gly-Ser-Gln-Arg-Pro-Arg-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-
OH (Kimura, T. et al, (1983) BBRC 114493; Fairwell, T. et al, (1983) Biochemistry 222691)

FIG.18

SEQ ID NO:16 - Rat PTH (rPTH) (84 amino acids)
H_2N-Ala-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ala-Ser-Val-Glu-Arg-Met-Gln-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ser-Leu-Gly-Val-Gln-Met-Ala-Ala-Arg-Glu-Gly-Ser-Tyr-Gln-Arg-Pro-Thr-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Asp-Gly-Asn-Ser-Lys-Ser-Leu-Gly-Glu-Gly-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Val-Lys-Ala-Lys-Ser-Gln-OH (Heinrich, G. et al, (1984) J. Biol. Chem. 2593320)

FIG.19

SEQ ID NO:17 - bPTH (1-34)
H_2N-Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH (Tregear, G. W. et al, (1977) Biochemistry 162817)

FIG.20

SEQ ID NO:18 - hPTH (1-34)
H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH (Takel, T. et al, (1979) Peptide Chemistry)

FIG.21

SEQ ID NO:19 - [Tyr^1] -hPTH (1-34)
H_2N-Tyr-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH

FIG.22

SEQ ID NO:20 - hPTH (1-38)
H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-OH (Heech, R. D. et al, (1984) Horm. Metab. Res. 16:556)

FIG.23

SEQ ID NO:21 - bPTH (3-34)
H_2N-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH (Lowrik, C. et al, (1985) Cell Calcium 6:311)

FIG.24

SEQ ID NO:22 - hPTH (13-34)
H_2N-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH

FIG.25

SEQ ID NO:23 - [Tyr^{27}]-hPTH (27-28)
H_2N-Tyr-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-Ala-Gly-Ser-OH

FIG.26

SEQ ID NO:24 - hPTH (28-48)
H_2N-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-Asp-Ala-Gly-Ser-OH Rosenblatt, M. et al, (1977) Biochemistry 16:2811)

FIG.27

SEQ ID NO:25 - hPTH (53-84)
H_2N-Lys-Lys-Glu-Asp-Asn-Val-Leu-Val-Glu-Ser-His-Glu-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asn-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-OH
(Rosenblatt, M. et al, (1978) Endocrinology 103:976)

FIG.28

SEQ ID NO: 26 - Oligo -
5'-AGCGGAGACGATGCAGCGGAGA-3'

FIG.29

SEQ ID NO: 27 - Oligo -
5'-AAGGGAGGCAGCTGAGACG-3'

FIG.30

SEQ ID NO: 28 - Oligo -
5'-GTCCTTGGAAGGTCTCTGCTG-3'

FIG.31

SEQ ID NO: 29 - Oligo -
5'-TTCTAGTGCCACTGCCCATTG-3'

FIG.32

SEQ ID NO: 30 - Oligo -
5'-CTACTAAGCTGTGTGGATTTCTGCGAT-3'

FIG.33

SEQ ID NO: 31 - hPTHrP- (1-34) -
AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTAE

FIG.34

SEQ ID NO: 32 - hPTHrP (1-141) -
AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRFGSDDEGR
YLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDSGVTGSGLEGD
HLSDTSTTSLELDSRRH

FIG.35

SEQ ID NO: 33 - hPTHrP (1-139) -
AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRFGSDDEGR
YLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDSGVTGSGLEGD
HLSDTSTTSLELDSR

FIG.36

SEQ ID NO: 34 - hPTHrP (1-173) -
Met-Gln-Arg-Arg-Leu-Val-Gln-Gln-Trp-Ser-Val-Ala-Val-Phe-Leu
Leu-Ser-Tyr-Ala-Val-Pro-Ser-Cys-Gly-Arg-Ser-Val-Glu-Gly-Leu
Ser-Arg-Arg-Leu-Lys-Arg-Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His
Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Phe-Phe-Leu
His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-Glu-Ile-Arg-Ala-Thr
Ser-Glu-Val-Ser-Pro-Asn-Ser-Lys-Pro-Ser-Pro-Asn-Thr-Lys-Asn
His-Pro-Val-Arg-Phe-Gly-Ser-Asp-Asp-Glu-Gly-Arg-Tyr-Leu-Thr
Gln-Glu-Thr-Asn-Lys-Val-Glu-Thr-Tyr-Lys-Glu-Gln-Pro-Leu-Lys
Thr-Pro-Gly-Lys-Lys-Lys-Lys-Gly-Lys-Pro-Gly-Lys-Arg-Lys-Glu
Gln-Glu-Lys-Lys-Lys-Arg-Arg-Thr-Arg-Ser-Ala-Trp-Leu-Asp-Ser
Gly-Val-Thr-Gly-Ser-Gly-Leu-Glu-Gly-Asp-His-Leu-Ser-Asp-Thr
Ser-Thr-Thr-Ser-Leu-Glu-Leu-Asp-Ser-Arg-Thr-Ala-Leu-Leu-Trp
Gly-Leu-Lys-Lys-Lys-Lys-Glu-Asn-Asn-Arg-Arg-Thr-His-His-Met
Gln-Leu-Met-Ile-Ser-Leu-Phe-Lys-Ser-Pro-Leu-Leu-Leu-Leu-End

FIG.37

SEQ ID NO: 35 - hPTH (7-34) -
LMHNLGKHLNSMERVEWLRKKLQDVHNF

FIG.38

SEQ ID NO: 36 - hPTHrP (7-34) -
LLHDKGKSIQDLRRRFFLHHLIAEIHTAE

FIG.39

SEQ ID NO: 37 - hPTH (5-36) -
IQLMHNLGKHLNSMERVEWLRKKLQDVHNFVA

FIG.40

SEQ ID NO: 38 - hPTHrP (5-36)
H_2N-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-
Phe-Phe-Leu-His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-Glu-Ile-OH

FIG.41

SEQ ID NO: 39 - hPTH (5-34)
H_2N-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-OH

FIG.42

SEQ ID NO: 40 - hPTHrP (5-34)
H_2N-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Phe-Phe-Leu-His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-OH

FIG.43

SEQ ID NO: 41 - hPTHrP (7-139) -
LLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRFGSDDEGR
YLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDSGVTGSGLEGD
HLSDTSTTSLELDSR

FIG.44

SEQ ID NO: 42 - hPTHrP (7-141) -
LLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRFGSDDEGR
YLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDSGVTGSGLEGD
HLSDTSTTSLELDSRRH

FIG.45

SEQ ID NO: 43 - hPTHrP (7-173) -
Gln-Gln-Trp-Ser-Val-Ala-Val-Phe-Leu

Leu-Ser-Tyr-Ala-Val-Pro-Ser-Cys-Gly-Arg-Ser-Val-Glu-Gly-Leu

Ser-Arg-Arg-Leu-Lys-Arg-Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His

Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Phe-Phe-Leu

His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-Glu-Ile-Arg-Ala-Thr

Ser-Glu-Val-Ser-Pro-Asn-Ser-Lys-Pro-Ser-Pro-Asn-Thr-Lys-Asn

His-Pro-Val-Arg-Phe-Gly-Ser-Asp-Asp-Glu-Gly-Arg-Tyr-Leu-Thr

Gln-Glu-Thr-Asn-Lys-Val-Glu-Thr-Tyr-Lys-Glu-Gln-Pro-Leu-Lys

Thr-Pro-Gly-Lys-Lys-Lys-Lys-Gly-Lys-Pro-Gly-Lys-Arg-Lys-Glu

Gln-Glu-Lys-Lys-Lys-Arg-Arg-Thr-Arg-Ser-Ala-Trp-Leu-Asp-Ser

Gly-Val-Thr-Gly-Ser-Gly-Leu-Glu-Gly-Asp-His-Leu-Ser-Asp-Thr

Ser-Thr-Thr-Ser-Leu-Glu-Leu-Asp-Ser-Arg-Thr-Ala-Leu-Leu-Trp

Gly-Leu-Lys-Lys-Lys-Lys-Glu-Asn-Asn-Arg-Arg-Thr-His-His-Met

Gln-Leu-Met-Ile-Ser-Leu-Phe-Lys-Ser-Pro-Leu-Leu-Leu-Leu-End

FIG.46

SEQ ID NO: 44 - hPTH (1-44)
H_2N-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe-Val-Ala-Leu-Gly-Ala-Pro-Leu-Ala-Pro-Arg-OH (Kimura T. et al, (1981) Biopolymers 20:1823)

FIG.47

SEQUENCE LISTING

<110> Holick, Michael F.

<120> Regulation of Cell Proliferation And Differentiation Using Topically Applied Nucleic Acid Molecules

<130> 1539.032PC00

<140>
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<150> US 60/238,134
<151> 2000-10-06

<160> 44

<170> PatentIn version 3.0

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<212> DNA
<213> Human Parathyroid Hormone Coding Sequence (hPTH)

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gtagaatggc tgcgtaagaa gctgcaggat gtgcacaatt ttgttgccct tggagctcct 120

ctagctccca gagatgctgg ttcccagagg ccccgaaaaa aggaagacaa tgtcttggtt 180

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gctaaatccc agtga 255

<210> 2
<211> 252
<212> DNA
<213> Bovine Parathyroid Hormone Coding Sequence (bPTH)

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atagcttaca gagatggtag ttcccagaga cctcgaaaaa aggaagacaa tgtcctggtt 180

gagagccatc agaaaagtct tggagaagca gacaaagctg atgtggatgt attaattaaa 240

gctaaacccc ag 252

<210> 3
<211> 426
<212> DNA
<213> Human Parathyroid Hormone Related Protein Coding Sequence (PTHrP)

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gaggtgtccc ctaactccaa gccctctccc aacacaaaga accaccccgt ccgatttggg 180

tctgatgatg agggcagata cctaactcag gaaactaaca aggtggagac gtacaaagag 240

cagccgctca agacacctgg gaagaaaaag aaaggcaagc ccgggaaacg caaggagcag 300

gaaaagaaaa aacggcgaac tcgctctgcc tggttagact ctggagtgac tgggagtggg 360

ctagaagggg accacctgtc tgacacctcc acaacgtcgc tggagctcga ttcacggagg 420

cattga 426

<210> 4
<211> 255
<212> DNA
<213> Rat Parathyroid Hormone Coding Sequence (rPTH)

<400> 4
gctgtcagtg aaatacagct tatgcacaac ctgggcaaac acctggcctc tgtggagagg 60

atgcaatggc tgagaaaaaa gctgcaagat gtacacaatt ttgttagtct tggagtccaa 120

atggctgcca gagaaggcag ttaccagagg cccaccaaga aggaggaaaa tgtccttgtt 180

gatggcaatt caaaaagtct tggcgagggg gacaaagctg atgtggatgt attagttaag 240

gctaaatctc agtaa 255

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<211> 30
<212> PRT
<213> hPTH (1-31)

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1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Leu Gln Asp Val
20 25 30

<210> 6
<211> 40
<212> PRT
<213> PTHrP - (1-40)

<400> 6
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1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala Glu Ile Arg Ala Thr Ser
35 40

<210> 7
<211> 84

<212> PRT
<213> bPTH

<400> 7
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1 5 10 15

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20 25 30

Asn Phe Val Ala Leu Gly Ala Ser Ile Ala Tyr Arg Asp Gly Ser Ser
35 40 45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Gln
50 55 60

Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asp Val Leu Ile Lys
65 70 75 80

Ala Lys Pro Gln

<210> 8
<211> 22
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<213> [Tyr^{63}] hPTH (63-84)

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Thr Lys Ala Lys Ser Gln
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<210> 9
<211> 21
<212> PRT
<213> hPTH (64-84)

<400> 9
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Lys Ala Lys Ser Gln
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<210> 10
<211> 16
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<210> 11
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<212> PRT
<213> hPTH (70-84)

<400> 11
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1 5 10 15

<210> 12
<211> 78
<212> PRT
<213> hPTH (7-84)

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Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu Gly
20 25 30

Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser Gln Arg Pro Arg Lys Lys
35 40 45

Glu Asp Asn Val Leu Val Glu Ser His Glu Lys Ser Leu Gly Glu Ala
50 55 60

Asp Lys Ala Asp Val Asn Val Leu Thr Lys Ala Lys Ser Gln
65 70 75

<210> 13
<211> 32
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<213> hPTHrP (1-31)

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20 25 30

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<211> 34
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<213> hPTH (1-34)

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Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
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Asn Phe

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<211> 84
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<213> hPTH

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1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser
35 40 45

Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu
50 55 60

Lys Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr Lys
65 70 75 80

Ala Lys Ser Gln

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20 25 30

Asn Phe Val Ser Leu Gly Val Gln Met Ala Ala Arg Glu Gly Ser Tyr
35 40 45

Gln Arg Pro Thr Lys Lys Glu Asp Asn Val Leu Val Asp Gly Asn Ser
50 55 60

Lys Ser Leu Gly Glu Gly Asp Lys Ala Asp Val Asp Val Leu Val Lys
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Ala Lys Ser Gln

<210> 17
<211> 34
<212> PRT
<213> bPTH (1-34)

<400> 17
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1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
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Asn Phe

<210> 18
<211> 34
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<213> hPTH (1-34)

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Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
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Asn Phe

<210> 19
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<213> [Tyr^1] hPTH (1-34)

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1 5 10 15

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
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Asn Phe

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<211> 38
<212> PRT
<213> hPTH (1-38)

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1 5 10 15

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Asn Phe Val Ala Leu Gly
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<210> 21
<211> 32
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<213> bPTH (3-34)

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1 5 10 15

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20 25 30

<210> 22
<211> 22
<212> PRT
<213> hPTH (13-34)

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1 5 10 15

Gln Asp Val His Asn Phe
20

<210> 23
<211> 22
<212> PRT
<213> [Tyr^{27}] hPTH (27-28)

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Tyr Leu Gln Asp Val His Asn Phe Val Ala Leu Gly Ala Pro Leu Ala
1 5 10 15

Pro Arg Asp Ala Gly Ser
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<210> 24
<211> 21
<212> PRT
<213> hPTH (28-48)

<400> 24
Leu Gln Asp Val His Asn Phe Val Ala Leu Gly Ala Pro Leu Ala Pro
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Arg Asp Ala Gly Ser
20

<210> 25
<211> 32
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<213> hPTH (53-84)

<400> 25
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1 5 10 15

Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr Lys Ala Lys Ser Gln
20 25 30

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<213> Oligonucleotide

<400> 26
agcggagacg atgcagcgga ga 22

<210> 27
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<400> 27
aagggaggca gctgagacg 19

<210> 28

<211> 21
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<213> Oligonucleotide

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<210> 29
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<212> DNA
<213> Oligonucleotide

<400> 29
ttctagtgcc actgcccatt g 21

<210> 30
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<400> 30
ctactaagct gtgtggattt ctgcgat 27

<210> 31
<211> 35
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1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala Glu
35

<210> 32
<211> 141
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<213> hPTHrP (1-141)

<400> 32
Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln
1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
20 25 30

Thr Ala Glu Ile Arg Ala Thr Ser Glu Val Ser Pro Asn Ser Lys Pro
35 40 45

Ser Pro Asn Thr Lys Asn His Pro Val Arg Phe Gly Ser Asp Asp Glu
50 55 60

Gly Arg Tyr Leu Thr Gln Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu
65 70 75 80

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Gln Pro Leu Lys Thr Pro Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys
                85                  90                  95

Arg Lys Glu Gln Glu Lys Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu
            100                 105                 110

Asp Ser Gly Val Thr Gly Ser Gly Leu Glu Gly Asp His Leu Ser Asp
        115                 120                 125

Thr Ser Thr Thr Ser Leu Glu Leu Asp Ser Arg Arg His
    130                 135                 140
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<213> hPTHrP (1-139)

<400> 33

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1               5                   10                  15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His
            20                  25                  30

Thr Ala Glu Ile Arg Ala Thr Ser Glu Val Ser Pro Asn Ser Lys Pro
        35                  40                  45

Ser Pro Asn Thr Lys Asn His Pro Val Arg Phe Gly Ser Asp Asp Glu
    50                  55                  60

Gly Arg Tyr Leu Thr Gln Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu
65                  70                  75                  80

Gln Pro Leu Lys Thr Pro Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys
                85                  90                  95

Arg Lys Glu Gln Glu Lys Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu
            100                 105                 110

Asp Ser Gly Val Thr Gly Ser Gly Leu Glu Gly Asp His Leu Ser Asp
        115                 120                 125

Thr Ser Thr Thr Ser Leu Glu Leu Asp Ser Arg
    130                 135
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<210> 34
<211> 209
<212> PRT
<213> hPTHrP (1-173)

<400> 34

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1               5                   10                  15

Ser Tyr Ala Val Pro Ser Cys Gly Arg Ser Val Glu Gly Leu Ser Arg
            20                  25                  30

Arg Leu Lys Arg Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly
        35                  40                  45
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Lys Ser Ile Gln Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile
50 55 60

Ala Glu Ile His Thr Ala Glu Ile Arg Ala Thr Ser Glu Val Ser Pro
65 70 75 80

Asn Ser Lys Pro Ser Pro Asn Thr Lys Asn His Pro Val Arg Phe Gly
85 90 95

Ser Asp Asp Glu Gly Arg Tyr Leu Thr Gln Glu Thr Asn Lys Val Glu
100 105 110

Thr Tyr Lys Glu Gln Pro Leu Lys Thr Pro Gly Lys Lys Lys Lys Gly
115 120 125

Lys Pro Gly Lys Arg Lys Glu Gln Glu Lys Lys Lys Arg Arg Thr Arg
130 135 140

Ser Ala Trp Leu Asp Ser Gly Val Thr Gly Ser Gly Leu Glu Gly Asp
145 150 155 160

His Leu Ser Asp Thr Ser Thr Thr Ser Leu Glu Leu Asp Ser Arg Thr
165 170 175

Ala Leu Leu Trp Gly Leu Lys Lys Lys Lys Glu Asn Asn Arg Arg Thr
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His His Met Gln Leu Met Ile Ser Leu Phe Lys Ser Pro Leu Leu Leu
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Leu

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20 25

<210> 36
<211> 29
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<213> hPTHrP (7-34)

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1 5 10 15

Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala
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<210> 38
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His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
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Thr Ser Glu Val Ser Pro Asn Ser Lys Pro Ser Pro Asn Thr Lys Asn
35 40 45

His Pro Val Arg Phe Gly Ser Asp Asp Glu Gly Arg Tyr Leu Thr Gln
50 55 60

Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu Gln Pro Leu Lys Thr Pro
65 70 75 80

Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys Arg Lys Glu Gln Glu Lys
85 90 95

Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu Asp Ser Gly Val Thr Gly
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<210> 42
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Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala Glu Ile Arg Ala
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35 40 45

His Pro Val Arg Phe Gly Ser Asp Asp Glu Gly Arg Tyr Leu Thr Gln
50 55 60

Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu Gln Pro Leu Lys Thr Pro
65 70 75 80

Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys Arg Lys Glu Gln Glu Lys
85 90 95

Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu Asp Ser Gly Val Thr Gly
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20 25 30

Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu

35 40 45

Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala
50 55 60

Glu Ile Arg Ala Thr Ser Glu Val Ser Pro Asn Ser Lys Pro Ser Pro
65 70 75 80

Asn Thr Lys Asn His Pro Val Arg Phe Gly Ser Asp Asp Glu Gly Arg
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Tyr Leu Thr Gln Glu Thr Asn Lys Val Glu Thr Tyr Lys Glu Gln Pro
100 105 110

Leu Lys Thr Pro Gly Lys Lys Lys Lys Gly Lys Pro Gly Lys Arg Lys
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Glu Gln Glu Lys Lys Lys Arg Arg Thr Arg Ser Ala Trp Leu Asp Ser
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Gly Val Thr Gly Ser Gly Leu Glu Gly Asp His Leu Ser Asp Thr Ser
145 150 155 160

Thr Thr Ser Leu Glu Leu Asp Ser Arg Thr Ala Leu Leu Trp Gly Leu
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Lys Lys Lys Lys Glu Asn Asn Arg Arg Thr His His Met Gln Leu Met
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Ile Ser Leu Phe Lys Ser Pro Leu Leu Leu Leu
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Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His
20 25 30

Asn Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg
35 40

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(25) Filing Language: English

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(30) Priority Data:
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(74) Agents: **ESMOND, Robert, W.** et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., 1100 New York Avenue, N.W., Suite 600, Washington, DC 20005-3934 (US).

(81) Designated States *(national)*: AU, CA, JP, KR, US.

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Published:
— *with international search report*

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(15) Information about Correction:
Previous Correction:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/028420 A3

(54) Title: REGULATION OF CELL PROLIFERATION AND DIFFERENTIATION USING TOPICALLY APPLIED NUCLEIC ACID MOLECULES

(57) Abstract: Methods are disclosed for the regulation of cell differentiation and proliferation, e.g., for treating hyperproliferative skin disorder, such as psoriasis, and skin cancer for enhancing wound healing, for stimulating hair growth and inhibiting hair growth, by administration of nucleic acid molecules encoding parathyroid hormone (PTH), parathyroid related peptide (PTHrP), or fragment, analog or derivative thereof, and salts thereof, encapsulated by particular liposomes or incorporated into a porous boicompatable matrix.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/31082

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/29 A61K31/7088 A61K48/00 C12N5/08 A61K9/127
A61K7/06 A61K7/48 A61P17/00 A61P35/00 A61K38/17
A61K38/00 A61K38/24 //(A61K38/29,31:59)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 914 126 A (LI LINGNA ET AL) 22 June 1999 (1999-06-22)	1-3,5-7, 10,11, 29-35, 37,39 41
Y	column 5, line 4 - line 31 column 1, line 61 - line 62 column 22, line 21 - line 25	
Y	US 6 066 618 A (HOLICK MICHAEL F) 23 May 2000 (2000-05-23) column 3, line 25 -column 4, line 60	1-11, 18-20, 29-39

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 March 2003	02 04 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Bayrak, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	HOLICK M F ET AL: "A parathyroid hormone antagonist stimulates epidermal proliferation and hair growth in mice" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 91, no. 17, August 1994 (1994-08), pages 8014-8016, XP002213035 ISSN: 0027-8424 the whole document	1-11, 18-20, 29-39
Y	WO 97 38729 A (UNIV MICHIGAN) 23 October 1997 (1997-10-23) page 16 page 41, line 6 - line 37	1-11, 18-20, 28-39
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INTERNATIONAL SEARCH REPORT

ational application No.
PCT/US 01/31082

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-11, 18-20,36 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: claims 1-41 (all partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11,18-20,36-38

Claims 1-11,18-20, 36-38: inhibition of proliferation/enhancing differentiation of a mammalian skin or hair cell by the administration of a proliferation inhibiting/differentiation enhancing nucleic acid molecule, such as defined in claim 1.

2. Claims: 29-35,39

Claims 29-35,39: induction of proliferation of a mammalian skin or hair cell by the administration of a proliferation inducing nucleic acid molecule, such as defined in claims 29.

3. Claims: 12-17,21-28,40,41

Claims 12-17,21-28,40,41: inhibition of proliferation/enhancing differentiation of a mammalian skin or hair cell by the administration of a proliferation inhibiting/differentiation enhancing nucleic acid molecule, such as defined in claim 1, and an active vitamin D compound.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: claims 1-41 (all partially)

1. Claims 1-4,7-10,12-34,37-41 relate to compounds defined by reference to a desirable characteristic or property, namely "..a nucleic acid molecule, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and is capable of inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or in vivo in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth" or "..a nucleic acid molecule, wherein the peptide encoded by the nucleic acid molecule is at least 3 amino acids long, has at least 10% sequence identity with the 34 amino acid N-terminal region of hPTH or hPTHrP, and is capable of blocking the inhibition of proliferation or stimulation of differentiation in vitro of cultured human keratinocytes by ...inhibiting proliferation or enhancing differentiation in vitro of cultured human keratinocytes, or in vivo in mouse skin by inhibiting skin cell proliferation or hair cycle progression or hair cell growth". The claims 1-4,7-10,12-34,37-41 cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity because the above expressions are unclear and leave the reader in doubt as to the meaning of the technical feature to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT). Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

2. Claims 1-8,11-32,35-41 relate to the use of a pharmaceutical preparation for the treatment of "hyperproliferative skin disorder", "inhibition of proliferation/enhancing differentiation of a mammalian skin or hair cell", or "inducing proliferation of a mammalian skin or hair cell" which is a generic term for a multitude of different diseases. The claim thus covers a multitude of diseases, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such diseases. Consequently, the claims lack support and the application lacks disclosure. Independent of the above reasoning, claims 1,8,37 also lacks clarity because it is not fully possible to determine the diseases for which protection might legitimately be sought (Article 6 PCT).

Consequently, the search for the first subject has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely nucleic acid molecules as disclosed in claims 5, 6, and 35 in relation to the treatment of diseases as defined in claims 9,10,33,34; and with due regard to the general concept of the invention.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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